A STUDY ON SECONDARY BACTERIAL INFECTIONS ASSOCIATED WITH DERMATOLOGICAL LESIONS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

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M.D.(MICROBIOLOGY)

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CERTIFICATE

This is to certify that this dissertation titled "**A STUDY ON SECONDARY BACTERIAL INFECTIONS ASSOCIATED WITH DERMATOLOGICAL LESIONS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **DR. S.VINOTHA,** during the period of her Post Graduate study from 2012 to 2015 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai- 600003, in partial fulfillment of the requirement of **M.D MICROBIOLOGY** degree Examination of The Tamilnadu Dr. M.G.R Medical University to be held in April 2015.

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DECLARATION

I declare that the dissertation entitled **" A STUDY ON SECONDARY BACTERIAL INFECTIONS ASSOCIATED WITH DERMATOLOGICAL LESIONS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL**" submitted by me for the degree of M.D. is the record work carried out by me during the period of **September 2013 – August 2014** under the guidance of **Dr. S.Vasanthi, M.D.,** Professor, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical Unversity, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Branch IV (Microbiology) examination to be held in April 2015.

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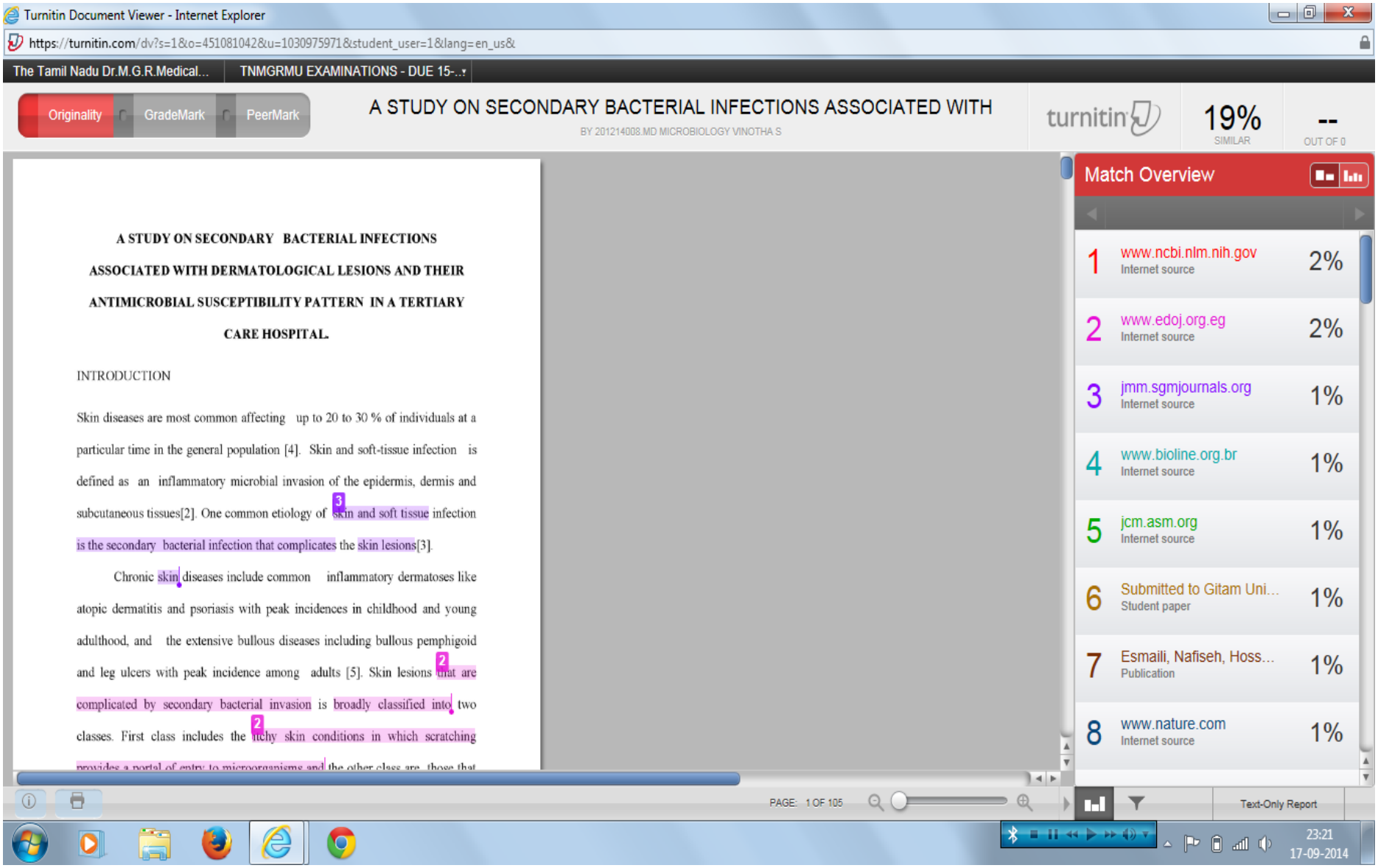
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INTRODUCTION
Skin diseases are most common affecting up to 20 to 30 % of individuals at a particular time in the general population [4]. Skin and soft-tissue infection is defined as an inflammatory microbial invasion of the epidermis, dermis and subcutaneous tissues[2]. One common etiology of skin and soft tissue infection is the secondary bacterial infection that complicates the skin lesions[3].
Chronic skin diseases include common inflammatory dermatoses like atopic dermatitis and psoriasis with peak incidences in childhood and young adulthood, and the extensive bullous diseases including bullous pemphigoid and leg ulcers with peak incidence among adults [5]. Skin lesions that are complicated by secondary bacterial invasion is broadly classified into two classes. First class includes the itchy skin conditions in which scratching
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A STUDY ON SECONDARY BACTERIAL INFECTIONS ASSOCIATED WITH DERMATOLOGICAL LESIONS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL

ABSTRACT

INTRODUCTION:

Skin and soft-tissue infection is defined as an inflammatory microbial invasion of the epidermis, dermis and subcutaneous tissues. One common etiology of skin and soft tissue infection is the secondary bacterial infection that complicates the skin lesions.

The dose, route and duration of the antimicrobial treatment in these patients are predicated with the knowledge of the potential pathogens with their antimicrobial sensitivity .

This study was conducted at the Institute of Microbiology ,Madras Medical college, Chennai , to isolate the pathogens from patients with secondarily infected skin lesions (Psoriasis, Atopic dermatitis, Pemphigus lesions and Leprosy with infected ulcer) from various sites and to determine their antimicrobial susceptibility pattern .

MATERIALS &METHODS:

200 patients (≥18 yrs) with signs and symptoms of secondary infection of skin lesions attending the department of Dermatolgy , Rajiv Gandhi Government General Hospital, Chennai were included in the study. Pus and blood samples were collected from these patients and processed by standard microbiological techniques.

RESULT:

Out of 200 samples 63 , 62,52 and 23 samples were taken respectively from Atopic Dermatitis/Eczema, Pemphigus, Psoriasis and Leprosy with infected ulcer ,including 114 inpatiens and 86 outpatients.88% of samples were positive for culture.

Aerobic gram positive organisms accounts for 59.9% followed by aerobic gram negative 37.27% and anaerobic organisms 2.83%. In Atopic Dermatitis/Eczema 57.7% of isolates were *Staphylococcus aureus* followed by *Streptococcus pyogenes*, Enterobacteriacea and anaerobic organisms.

Most common isolate in Psoriasis was *Staphylococcus aureus* (64%) followed by Enteric gram negative bacilli and *Staphylococcus epidermidis*.

Pseudomonas aeroginosa (48%) was the most common organism in leprosy with infected ulcer followed by *Proteus vulgaris*(22%).

Blood culture from17 In patients, [11 from Pemphigus(1.77%) and 6 from Psoriasis(1.15%)] resulted in MRSA isolation from two cases of Pemphigus.

ESBL producers were 62.5%. All the ESBL producers were sensitive to Imipenem.

64%*Staphylococcus aureus* were MSSA and 36% were MRSA. All MRSA were positive for *mec A* gene .MRSA from OP patients(CA MRSA) showed higher positivity for *Pvl* gene 90% and 22% of MRSA from IP patients(HA MRSA) were positive for Pvl gene. All the Methicillin resistant *Staphylococcus aureus* were sensitive to vancomycin.

CONCLUSION:

MRSA with higher rate of resistance to many routinely used antibiotics and Enterobacteriaceae with higher levels of ESBL production were isolated. Hence bacterial culture and sensitivity of specimens from the secondarily infected skin lesions should be performed to confirm the bacterial etiology and to initiate effective antibiotic treatment so as to decrease the morbidity and mortality of these patients, that also limits the misuse of antimicrobials which would prevent the emergence of resistant bacterial strains in the hospital and the community.

INTRODUCTION

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Chronic skin diseases include common inflammatory dermatoses like atopic dermatitis and psoriasis with peak incidences in childhood and young adulthood, and the extensive bullous diseases including bullous pemphigoid and leg ulcers with peak incidence among adults [5]. Skin lesions that are complicated by secondary bacterial invasion is broadly classified into two classes. First class includes the itchy skin conditions in which scratching provides a portal of entry to microorganisms and the other class are those that are characterized by the absence of skin barrier, like eczema, pemphigus and ulcers.[6]

Human skin by acting as a physical barrier, secreting low pH sebaceous fluid and fatty acids functions as a first line of defence against micro organisms. It also has the normal flora mainly bacterial, which decreases the colonization by pathogenic organisms . An intact

stratum corneum prevents invasion of skin by normal skin flora or pathogenic microorganisms[1]. These barriers are lost most commonly in the Skin and soft-tissue infections(SSTIs).

The normal healthy skin surface is colonized by many bacterial species like *Staphylococcus aureus, diphtheroids* and *coagulase negative staphyloccocci* which under normal conditions do not lead to cutaneous infections. But, when the skin barrier function is disturbed by a chronic skin disease there will be a massive microbial colonization that subsequently leads to clinically apparent cutaneous infection[4].

Staphylococcus aureus accounts for 30-50% of skin and soft tissue infections, followed by the enterobacteriaeceae, non-fermenters, *beta-hemolytic Group A streptococc*i and anaerobes. There is also a high risk of colonization with *Staphylococcus aureus* and cutaneous infections among patients with chronic skin lesions like atopic dermatitis, psoriasis. *Staphylococcus aureus* are found in 60% of psoriasis patients, and 88% of atopic dermatitis patients[8]. On an average more than 90% of community acquired strains of *Staphylococcus aureus* elaborate penicillinases or beta-lactamases and 20-30% of *Staphylococcus aureus* are methicillin resistant. The prevalence of MRSA in India also shows a rise in trend and there are reports of MRSA in the community-acquired infections also though the prevalence is much lesser. MRSA strains also

shows a high degree of resistance to other antibiotics especially, erythromycin and aminoglycosides [16].

Hence patients with chronic skin diseases may have an increased risk of more severe cutaneous infections, and needs prolonged period of antibiotic treatment. These infections may lead to serious local and systemic complications which progress rapidly and could be potentially life- threatening .

The dose route and duration of the antimicrobial treatment is predicated with the knowledge of the potential pathogens with their antimicrobial sensitivity , disease severity and clinical complications. Hence the recognition of the resistance of these organisms can help in guiding appropriate selection of antibiotic therapy.

This cross sectional study was done to isolate the bacteria from patients attending the dermatology department of Rajiv Gandhi Government General Hospital with various secondarily infected skin lesions (Psoriasis, Atopic dermatitis, Pemphigus lesions and Leprosy with infected ulcer) from various sites and to determine their antimicrobial susceptibility pattern so as to initiate effective antibiotic therapy thereby decreasing the morbidity and mortality of the patients.

AIMS AND OBJECTIVES

1. To isolate the bacteria associated with dermatological lesions and to determine their antimicrobial susceptibility pattern.

2. To study the bacteriological profile of various chronic skin lesions (Atopic dermatitis/Eczema, Psoriasis, Pemphigus, Leprosy with infected ulcer).

3. To study the isolation of pathogens from various anatomical sites.

4. To determine the antimicrobial resistance pattern of the most commonly isolated organism by phenotypic and genotypic methods.

5. To evolve an antibiotic policy for the management of these infections.

REVIEW OF LITERATURE

The firm knowledge that bacteria were the causative and transmitting agent of disease and were responsible for contagion was acquired in the 19th century, but the idea that there were tiny creatures that could produce illness has been held for thousands of years. The ideas of infection were recorded by Hippocrates in 300BC and proposed in his classic tome "De Contagione" that "seeds of contagion" might be responsible for infection[9].

PREVALENCE OF SECONDARY BACTERIAL INFECTION IN CHRONIC SKIN LESIONS:

Secondary bacterial infections are common complications of primary dermatoses like Dermatitis/Eczema, Psoriasis, Pemphigus, chronic ulcers of Leprosy. Prevalence of infection varies between the lesions. Infection is most commonly caused by mixed bacterial flora whose origin is endogenous oral, gastrointestinal or skin [10, 11]. Colonization means that the bacteria grow and multiply on the nutrient surface of the skin without clinical apparent infection, and infection means that multiplication within the skin leading to clinical apparent infection within the skin or deeper. *Staphylococcus aureus* was found in 60% of psoriasis patients, 88% of atopic dermatitis patients[4]. In

pemphigus the most common isolates are *Staphylococcus aureus* which accounts for 68% of infection followed by Enterobacteriaceae [13].In leprosy with infected ulcers the most common isolates were *Pseudomonas* and *Proteus* followed by *Staphylococcus*[14,15].

NORMAL CUTANEOUS FLORA

The organisms that survives and multiply in various ecologic niches of skin constitutes the "normal cuaneous flora".Normal skin flora can be classified into the following types[17].

Resident Flora:-

These organisms grow on the skin and are relatively stable in number and composition at a particular site and are attached to the skin. The increase in their number results from their multiplication and not due to addition of bacteria from outside.

Transient Flora:-

These organisms lies free on the skin without attachment and they are derived from exogenous sources. They are unable to multiply on the skin and vary both in type and number and disappear from skin with in a short time.

Temporary Residents/ transient Residents:-

These organisms can colonize the skin of a small percentage of subjects in a modest number for a little longer time[18].

PROTECTIVE MECHANISMS OF SKIN TO INFECTION:-

Intact skin acts as a physical barrier between the host, and the external world, preventing most pathogens from harming the host. The epidermis impedes penetration of microbial organisms, chemical irritants and toxins; absorbs and blocks solar and ionized radiation; and inhibits water loss.

The relative **dryness of normal skin** specifically contributes to the marked limitation of growth of bacteria; especially Gram negative bacilli. Humans have fewer bacteria on exposed parts such as hand and forearms than axilla[19].

Both innate immunity and adaptive immunity in skin are involved in protecting skin from invading organisms[20].

The innate immune system:-

- Innate immune system of skin relies on a series of "pattern recognition receptors" that recognize "pathogen-associated molecular patterns" that are not present on self. Binding of the

pattern recognition receptors to the pathogen-associated molecular patterns results in opsonization and activation of the complement system as well as induction of inflammatory signaling pathways. This process involves at least three pattern recognition receptors: (1) antimicrobial peptides (2) Toll-like receptors (TLRS), and (3) the complement system. All these three systems engage bacteria once they enter the skin and by signaling, bring neutrophils and other immune cells to the site of infection to destroy the pathogen[19,20].

Antimicrobial peptides :-

- Expressed on the skin surface as well as in eccrine sweat and saliva.
- Produced by activated keratinocytes and are delivered to the skin surface in the lamellar bodies. Their appearance on the skin surface is closely tied to the production of normal skin stratum corneum lipids.
- These small proteins have a characteristic physical property, the presence of an amphipathic organization, with one portion being cationic and capable of binding to microbial membranes, and another being hydrophobic allowing for insertion into bacterial lipid membrane. The insertion into the membrane results in membrane disruption and microbial death. A second principle of anti microbial

peptides is that they are processed after release by enzymes on the skin surface, resulting in multiple peptides each with different activities and different targets. The third principle of antimicrobial peptides is that they are also potent activators of the host immune response. The two antimicrobial peptides studied to date on the skin are the cathelicidines (LL-37) and the β-defensins.

Cathelicidines:-

- Peptides with a structurally variable antimicrobial domain at the C-terminus.

- Humans possess only one type of cathelicidine gene. The human precursor protein hCAP 18 (human cathelicidine antimicrobial protein 18) is produced by skin cells, including keratinocytes, mast cells, neutrophils, and ductal cells of eccrine glands.

- Neutrophil proteases (proteinase 3) process hCAP18 into effector molecule LL-37, which has antibacterial, antiviral and antifungal properties. LL-37 further contributes to innate immunity by attracting mast cells and neutrophils via formyl peptide receptor and by inducing mediator release from latter cells via a G protein-dependent, immunoglobulin E independent mechanism. It has now been shown that LL-37 is secreted into human sweat, where it is

cleaved by a serine protease-dependent mechanism into its peptides RK-31 or KS-30, which display an even more potent antimicrobial activity than intact LL-37.

β-Defensins

Cysteine-rich cationic low-molecular-weight antimicrobial peptides.

Three types (HBD-1, HBD-2 and HBD-3) of β -defensins were isolated.

HBD-1 is constitutively expressed on epidermis and has antimicrobial activity against Gram negative bacteria and appears to play a role in keratinocyte differentiation.

HBD-2 is inducible by microbes, including *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans,* and also by pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1. It has antimicrobial activity against Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* but not against Gram positive bacteria such as *Staphylococcus aureus*.

HBD-3 is induced by contact with tumor necrosis factor-α and certain pathogens. It has potent antimicrobial activity against

Staphylococcus aureus and vancomycin-resistant *Enterococcus faecium*, making it the first human β -defensin in skin to be effective against Gram positive bacteria.

Toll like receptors (TLRS):-

TLRs occur on the cell membranes and recognize certain exogenous ligands that are unique to invading microorganisms and not found in the host. They play a prominent role as primary sensors for invading pathogens. TLR5 recognizes flagellin, unique to flagellated bacteria, and TLR2 recognizes the peptidoglycan on the surface of Gram positive bacteria. TLRS also instruct antigen presenting cells that have engaged the organism to secrete appropriate cytokines to generate the desired immunologic milieu and eventual adaptive immune response.

Complement:-

Mannan-binding lectin binds to carbohydrate patterns on bacteria and activates C2 and C4. Activation of C3 liberates C3a and C3b. C3b on membranes leads to opsonization and enhanced phagocytosis. The cleavage of C5 leads to C5a, a potent activator of neutrophils and a stimulator of pro-inflammatory cytokines, including interleukin l(IL-l) and IL-8. The "membrane attack complex" is formed by completion of the complement cascade and kills invading microbes[I7].

Lipids:-

The free fatty acids, linoleic and linolenic acids are inhibitory for Staphylococcus aureus. Sphingosine, glucosylceramides, and cis-6-hexadeconic acid have been demonstrated to have antimicrobial activity against Staphylococcus aureus.

Resident flora, particularly the lipophilic *corynebacteria*, release lipases and thus contribute to defense against *Streptococcus pyogenes* and Staphylococcus aureus by liberating fatty acids from triglycerides of sebum. The acid mantle thus created favours the growth of propionibacterium, which in turn produce propionic acid; which has relatively more antimicrobial activity against transient organisms than resident flora.[21]

Bacterial interference:-

Bacterial interference is the suppressive effect of one bacterial species on the colonization by another, exerts a major influence on the overall composition of the skin flora. Normal skin is colonized with resident bacterial flora, usually *Staphylococcus epidermidis*, other *coagulase negative staphylococci, corynebacteria* and *Propionibacterium acnes*. These bacteria form a protective layer and prevent the adhesion and multiplication of potential pathogens by producing many inhibitory

products or by modifying skin secretion. *Staphylococcus aureus*, when applied on the skin does not survive long but when applied on skin pretreated with 70% ethanol, it colonizes indicating the effect of bacterial interference. Colonization of a site by one strain of bacteria interferes with subsequent colonization by another strain of bacteria, which may be due to competition for same nutrient or by production of antibiotics.[17]

Keratinocytes and other epidermal cells produce **reactive oxygen species,** which have potent inflammation inducing properties as well as immunomodulatory properties that act as an important host defense mechanism against microbial invasion.[17]

Desquamation of skin results in loss of the transient flora.[17]

Adaptive immune response in skin[19,20]

Dendritic antigen presenting cells in the epidermis and dermis initiates adaptive immune response in skin while T lymphocytes and antibodies execute it.

In skin, humoral immunity contributes to the immune defense against extracellular pathogens. Antibodies bind to microbial agents and neutralize them or facilitate uptake of the pathogen by phagocytes that destroy them. T lymphocytes contribute to cell-mediated immunity

(CMI), required to eliminate intracellular pathogens, by releasing cytokines.

SECONDARY BACTERIAL INFECTIONS IN SKIN LESONS:

Secondary infections can arise from the invasion of certain organisms from the external environment through the breaks in the skin. The dermatological lesions taken up in this study are AtopicDermatitis/AtopicEczema, Psoriasis, Pemphigus and Chronic infected ulcers of Leprosy.

1.Atopic Dermatitis/Eczema: Eczema also called as dermatitis which means inflammation of the skin. There are different types of eczema. The most common type is atopic eczema.It is more common in children and young adults[21].

Common signs and symptoms of atopic dermatitis (eczema) include:

Red to brownish-gray colored cracked or scaly skin lesions, itching, which may be severe, small raised bumps, which may ooze fluid and forms crusts over the lesions which then leads to thickening of the skin Lesions of a topic dermatitis most commonly occurs in the folds of the elbows, backs of the knees or the front of the neck. It tends to flare periodically and then subside after a particular time[22].

The presence of pustules, a purulent discharge,crusting combined with weeping crusting alone , or sudden appearance of weeping, are taken as physical signs of infection[24].

In eczema with secondary bacterial infection there is a typical pattern of skin inflammation which is responsible for the symptoms. The severity of the eczema and S*taphylococcus aureus* colonization has been demonstrated, and it has been shown that bacterial colonization is an important factor aggravating skin lesions[27].

In most patients with atopic dermatitis, even though there is an absence of skin lesions, colonization of *Staphylococcus aureus* will be noticed due to the altered immunological profile of atopic patients. Endogenous antimicrobial peptides i,e β-Defensins and Cathelicidines are under expressed in Atopic dermatitis. Clinical signs of impetiginization, such as weeping and crusting, or small superficial pustules are all a sensitive indicator that indicates the numbers of *Staphylococcus aureus* may have increased and is a clinical indication of secondary infected dermatitis. But the recent research that has focussed on the role of *Staphylococcus aureus* in atopic dermatitis, offers a reversed perspective, by presenting evidence that the underlying pathology of atopic dermatitis, i.e. an alteration of the skin barrier and inflammation of the upper dermis, depends itself on the presence of an infectious process.

In other words, secondary infection with *Staphylococcus aureus* emerging as a cause of atopic dermatitis. Recent research has greatly contributed to the understanding of the pathophysiological role of *Staphylococcus aureus* superantigens in atopic dermatitis, suggesting that antibiotic therapy might be an important element in the therapeutic management of atopic dermatitis[25,26].

2. Psoriasis:

It is a chronic lifelong skin disease most commonly causing erythematous papular and scaly plaques depending on lesion type.

According to Gudionsson, E. J. et al. (2003); and Guo-li, et al. 2009,[98] Psoriasis is a chronic immunologically mediated inflammatory disease of the skin and joint, which has been found to affect 1-3% of population. The exact etiology is unknown, but researchers believe heredity, environment and immune system also play a role in psoriasis.

Several clinical types of disease have been identified but the chronic plaque form Psoriasis vulgaris is the most common type as per Gudion sson, E. J. et al. (2003). and Mallbris, L. et al. (2005).

Psoriasis is a T-lymphocyte mediated inflammatory skin disease considered to have an autoimmune etiology.

The link between psoriasis and infection is most probably explained by the superantigen theory, that is superantigens are the products of bacteria, viruses, or fungi, which can bypass normal immunological pathway and leads to powerful stimulation to the immune system. According to Beaker B.S. et al. (2006) M protein carried by Streptococcus pyogenes acts as superantigen in provoking psoriasis.

Incidence of Psoriasis was found higher in in the age group of (18-40) years and majority of them (48.7%) were showing psoriatic lesions distributed whole over the body. *Staphylococcus aureus* is the most common cause of secondary infection (29.5%) followed by Proteus spp. and *Staphylococcus epidermidis* . Others like *Pseudomonas aeruginosa*, *Bacillus spp* are very rare[27].

3.Pemphigus:

Pemphigus is an autoimmune intra epidermal blistering lesion of the skin and mucous membrane .The term is derived from Greek word pemphix for blister or bubble[28,29].

Pemphigus vulgaris is the most common subtype of pemphigus group of disorders, which presents as flaccid mucocutaneous blisters and have a tendency to rupture easily followed by pemphigus foliaceus.

Pemphigus vulgaris (PV) and Pemphigus foliaceus (PF) are the organ-specific autoimmune bullous diseases characterized by loss of cell adhesion (acantholysis) and blister formation. These dermatoses are proved to be induced by autoimmune phenomenon. Considering this etiology, immunosuppressive therapies are the mainstay in the treatments available for these disorders. Infections are important complications in these patients attributable to disruption of the epidermal barrier because of the disease itself and immunosuppression induced by treatment.

There are many reports regarding predisposition to infections due to immunosuppressive therapy and the immunocompromised state of the pemphigus patients [33,34,35]. If left untreated, progression of the disease may lead to death within five years of the onset , due to secondary bacterial infection and sepsis.

Bullous pemphigoid is another most common autoimmune blistering skin disease and presents with large, tense, cutaneous blisters. Rupture of these bullae produce erosions that are susceptible to bacterial infection[30,31].

The most common causes of mortality in pemphigus are septicemia and pulmonary embolism; Septicemia usually follows cutaneous *Staphylococcus aureus* infection.

Before the advent of antibiotic therapy and corticosteroids pemphigus vulgaris caused a substantial mortality. Steroids, antibiotics and immunosuppressive agents have drastically improved the prognosis[36].

4. Chronic infected ulcers of Leprosy

Leprosy is a chronic infectious disease caused by the obligate intracellular pathogen *Mycobacterium leprae* . The bacteria grow best around 30°C thus have preference for the cooler areas of the human body[37]. Leprosy remains a public health problem, mainly in Africa, Asia and Latin America[38].

Complications of Leprosy are leprosy reactions, development of plantar and palmar ulcerations, lagophthalomus (failure of eyelids function) and corneal anesthesia. Leprosy is not itself directly responsible for many of the complications. It impairs the sensation of pain and hence exposes patients to ulceration and consequently to deformity.

Chronic ulcers are the most serious complications of leprosy and these ulcers are highly infected with bacteria, which is responsible for the delay in healing process. The delay is because of the competition between host cells and bacterial cells for oxygen and nutrients and also

the increased host cell production of inflammatory cytokines and proteases in response to the bacteria and their associated toxins.

Pseudomonas aeruginosa is the most common organism followed by staphylococci in Indian leprosy patients with ulcers[42].

There are absolute and relative indications for the use of antibiotics in leprosy ulcers. The absolute conditions are life threatening infective indications like septicemia and highly virulent bacterial infections such as staphylococci and streptococci. The relative indications include the presence of complications like cellulitis, acute regional lymphadenopathy, systemic toxaemia and involvement of deeper structures like underlying bones, joints or tendon sheaths[43,44].

Diagnosis of Secondary Bacterial infections:

Diagnosis is by examining the clinical signs and symptoms of infection followed by their culture and sensitivity. *Staphlococcus aureus* is the most common cause of secondary infection in many lesions, followed by Enterobacteriacea ,*Group A beta haemolytic streptococci* .

Pathogenesis of staphylococcal infection: [45]

Virulence factors of *Staphlococcus aureus*:

Cell wall components:

A.Capsule

Decreases chemotaxis and phagocytosis, decreases proliferation of mononuclear cells,facilitates adherence to foreign bodies.

B.Peptidoglycan

Maintains osmotic stability,stimulates production of endogenous pyrogen (which have endotoxin like activity,leucocyte chemoattractant (abscess formation) and decreases phagocytosis.

C.Teichoic acid

Regulates the cationic concentration at cell membrane and binds to fibronectin.

D.Protein A

Inhibits antibody mediated clearance by binding IgG1,IgG2 and IgG4 Fc receptors,leucocyte chemoattractants and anticomplementary.

E.Cytoplasmic membrane:

It acts as an osmotic barrier,regulates transport in and out of the cells and is a site of biosynthetic and respiratory enzymes.

TOXINS:

A.Cytotoxins: α, β, γ, δ and Panton Valentine Leukocidin: These are toxic for many cells including leucocytes, erythrocytes, macrophages, platelets and fibroblasts.

B.Exfoliative toxin (ETA ,ETB):Serine proteases which splits the intercellular bridges in the stratum granulosum of epidermis.

C.Enterotoxins:(A-E,G-I) These are Superantigens that stimulates the proliferation of T-cells and release of cytokines and inflammatory mediators in the mast cells.

D.Toxic Shock syndrome toxin I:Super antigen produce leakage and cellular destruction of endothelial cells

Enzymes:

A. Coagulase:Converts fibrinogen to fibrin.

B. Catalase:Catalyzes removal of H2O2 .

C. Hyaluronidase; Hydrolyzes hyaluronic acid in connective tissue, promoting the spread of staphylococci in tissue

D. fibrinolysin:Dissolves fibrin clots

E. Lipases: Hydrolyzes lipids

F. Nucleases:Hydrolyzes DNA

G. Penicillinase:Hydrolyzes penicillin

Role of Staphylococcus in Skin infections:[19,46]

For the effective invasion of the host ,the microbe must initially gain access. *Staphylococcus aureus* colonization may be transient or prolonged.Host Factors predisposing for staphylococcus infections are Atopy as in atopic dermatitis, Immunosuppression , preexisting tissue injury, and inflammation as in Pemphigus, Chronic ulcers of Leprosy and Psoriasis respectively.

Staphylococcus utilizes teichoic acid and other surface proteins that promotes the adherence to nasal mucosa, which then contaminate the breaches in the skin.

It also secretes many specific substances that attack the components of innate immunity system of skin.

Staphylokinase (SAK) inactivates defensins and activates plasminogen to plasmin. Surface plasmin cleaves C3b and immunoglobulin G, removing important opsonic molecules from bacterial surface. Chemotaxis inhibitory protein of Staphylococcus aureus binds to C5a,there by blocking neutrophil chemotaxis and activation.

Staphylococcal complement inhibitor binds to C3 convertase on the bacterial surface preventing it from C3 and complement cascade activation.

Yellow pigment of *Staphylococcus aureus*(carotenoids)protect it from oxidative killing by neutrophils.

Some strains produce one or more exotoxins like Staphylococcal enterotoxins,exfoliative toxins and leukocidin which inhibits the host immune response by their biological effect. Staphylococcal enterotoxin and TSS Toxin I acts as a super antigen,which produces massive non specific T cell activation and release of cytokines like Interleukin 1&2,interferonγ and Tumor necrosis factor α &β. Superantigen activation of T-cells also result in activation and expansion of lymphocytes expressing specific T cell receptor variable region of β chain.It may also activate B cells leading to high levels of IgE or autoantibodies.

Mode of infection:

Moist skin of anterior nares of 20-40 % adults,intertrigenious skin folds,axilla,vagina and perineum of healthy person horbours Staphylococcus aureus.

It may be exogenous, from direct contact, air borne or cross infection in hospitals or endogenous from colonization.

Diagnosis of staphyloccal infection:[47]

Specimens include pus and exudates from infected lesions and Blood if there are signs of sepsis.

Direct gram stain followed by culture, biochemical reactions and antibiotic sensitivity as per CLSI guidelines.

Antimicrobial resistance in Staphylococcus aureus:

Penicillin resistance: [48]

Penicillin resistance has been increasingly recognized since 1945.Nearly 80% or more strains of *Staphylococcus aureus* are resistant to penicillin.It is of 3 types.

1. Plasmid mediated resistance:

It is due to the production of the enzyme penicillinase (beta lactamase mediated by plasmids. The enzyme inactivates penicillin by splitting the beta lactam rings. *Staphylococcus aureus* produce 4 types of penicillinase (A, B, C, D). These plasmids are transmitted to Staphylococci by transduction and conjugation. The plasmid also carry resistance to other antibiotics like erythromycin and fusidic acid.

2. Chromosomal mediated resistance:

Reduction in the affinity of penicillin binding protein on the cellwall also plays a role in mediating resistance to penicillin and other beta lactam antibiotics.

3. Tolerance to penicillin:

Staphylococci developing tolerance to penicillin are only inhibited but not killed.

Methicillin resistance:

Methicillin resistance *Staphylococcus aureus* (MRSA) are resistant to all available penicillins and other beta lactam antibiotics. Resistance to methicillin indicates resistance to all cephalosporins. Many MRSA isolates are resistant to other antimicrobial families, including aminoglycosides, quinolones and macrolides. The first outbreak of MRSA infection occurred in European hospitals in 1960.From then there was a steady increase in occurance of MRSA infection and now it appears to be a worldwide phenomenon[49].

The prevalence of MRSA has shown an increasing trend in India.In 1996, Pulimood from Vellore reported 24%[50].The following year Udaya Shankar from Pondicherry reported 20%.

In 2006, Rajaduraipandi reported 37.9% from Coimbatore[50,51,52]. A study conducted by INSAR group ,showed that the prevalence of MRSA in our country is about 40 %[63].

The source of MRSA may be community acquired or hospital acquired. The latter might be from infected patients or hospital staff. The CDC definition of community acquired MRSA (CA-MRSA)is any MRSA infection diagnosed from an out patient or within 48 hrs of hospitalization, if the patient lacks the following healthcare associated risk factors, haemodialysis, surgery, long term hospitalization during previous year, presence of indwelling catheter or percutaneous device at the time of culture or previous isolation of MRSA from the patient. All others were considered to be hospital acquired MRSA(HA-MRSA)[49].

Mechanism of resistance

Mediated by mecA gene which encodes for penicillin binding protein2a(PBP2a) that has low affinity for beta lactams. mecA is carried on a mobile genetic element The Staphylococcal cassette chromosome (SCCmec).

Five types of SCCmec have been reported

Type I, II, III – HA – MRSA

Type IV a-d and V, Panton Valentine leukocidin (PVL)- with subunits lukS - PV and lukF PV – CA - MRSA.

They are integrated in *Staphylococcus aureus* genome at3'end of an open reading frame.

The genetic difference between HA-MRSA and CA-MRSA is the presence of a bacteriophage (phiSLT) carrying the pvl gene in CA-MRSA[53].

PVL is a bicomponent, pore forming leukotoxin initially called 'substance leukocidine' by Van de Velde in 1894 because of its ability to lyse leuckocytes. Panton and Valentine first associated the leukotoxin in 1932 before MRSA was of clinical concern with severe skin and soft tissue infections and necrotizing pneumonia among CA-MSSA and subsequently among CA-MRSA isolates [53].

The high virulence of CA-MRSA is associated with this PVL gene which mediate tissue necrosis and sepsis by either release of cytotoxic lysosomal granule contents from lysed Polymorphonuclear leukocytes or by an inflammatory cascade or by apoptosis.PVL is associated with

epidemic CA-MRSA strains causing skin infections. Most CA-MRSA infections are skin and soft tissue infections.This epidemic has made beta lactams which were previously uniformly effective against CA-MRSA isolates now becomes unreliable.

Other mechanisms of methicillin resistance:- [91]

Some strains of Staphylococcus aureus are not intrinsically resistant to methicillin and lack mecA and PBP2a.

BORSA (Border line Resistant Staphylococcus aureus) are less susceptible to methicillin because of hyper production of normal penicillinase.

MODSA (Methicillin Intermediate Staphylococcus aureus) show methicillin resistance due to other mechanisms and have normal PBP.

Both these groups are genetically distinct from MRSA and of unknown clinical and epidemiological importance though their infections can be effectively treated with beta lactamase resistant penicillins and cephalosporins.

Detection and identification of MRSA:

MRSA can be detected by both phenotypic and genotypic methods,The ideal method for identification is by detection of mecA gene

or its product PBP2a. But because of the high cost and requirement of exepertise it is not performed in most clinical laboratories and phenotypic identification of intrinsic methicillin resistance is the standard method followed.

A strain of Staphylococcus aureus is considered resistant to methicillin if the minimum inhibitory concentration(MIC) of oxacillin is≥4µg/ml[64].Oxacillin is preferred as it is more stable than methicillin.

Methods of identification of MRSA:[65]

1. Screening methods: with cefoxitin / oxacillin disc by disc diffusion method.

2. Confirmatory methods:

   Oxacillin MIC detection (by broth dilution, agar dilution, E test method),Oxacillin screen agar.

3. Molecular methods: detection of Mec A gene or PBP2aprotein(its protein product). [66,67]

Other methods are

MRSA screen Latex tests, Evigene MRSA kit Chromogenic agar

i) MRSA Select(Bipo-rad)

ii) Chrome Agar MRSA(Bio connections).

Typing methods for MRSA:

1. Biotyping:

It is a method to characterize MRSA based on biochemical and morphological properties.[68].Based on the following 4 properties

Tween 80 hydrolysis

Pigment production on Tween 80 agar

Urease production

Gentamicin resistance

Based on the result MRSA isolates have been divided into 4 groups(A,B,C.D)

All MRSA isolates were classified into four biotypes in the following way:

TEST	BIOTYPE			
	A	B	C	D
Tween 80 hydrolysis	–	–	+	+
Urease	–	+	–	+
Pigmentation	Cream	Buff	Variable	Gold
Gentamycin resistance	R	R	S	R

In India Biotyping by this technique was done for the first time in 1993 by Krishna Prakash S and showed that majority belongs to group B. He reported the same finding a decade later also.Similar finding were found by other author's also[69,70]. Since this technique is easy to perform ,inexpensive and reproducible, in can be incorporated as a daily bench top procedure.

2. Antibiogram:

MRSA can also be typed based on the susceptibility to a range of antibiotics. It is easy to perform but has a poor discriminatory ability and lacks reproducibility.

3.Genotypic methods:[66,67]

Plasmid analysis

Chromosomal DNA

Restriction enzyme analysis

Southern hybridization

Ribotyping

Coagulase gene typing

Protein A gene typing

RAPD

Rep-PCR

Mec-A:Tn 554 probe typing

Pulse- field gel electrophoresis

Resistance to other antibiotics:

Erythromycin and clindamycin:

These two are two different classes of antimicrobial agents the inhibit protein synthesis by binding to 50S ribosomal unit of bacterial cell. In Staphylococci resistant to both these drugs occur through methylation of their ribosomal target site. Such resistance is mediated by the msr A. Another mechanism of resistance is by inactivation of lincosamides by chemical modification, which is mediated by inu A gene.

The target site modification mechanism also called macrolide lincosamide-streptograminB(MLSB) resistance results in resistance to erythromycin, clindamycin and streptograminB.This may be constitutive or inducible. In constitutive rRNA methylase is always produced,whereas in inducible methylase is produced only in the presence of an inducer.

Invitro, Staphylococcus aureus isolates with constitutive resistance are resistant to erythromycin and clindamycin and isolates with inducible resistance are resistant to erythromycin but appear susceptible to clindamycin and invivo therapy with clindamycin may select for constitutive erm mutants ,and leads to clinical failure.

Invitro induction test can distinguish inducible erm –mediated resistance from those with msr-A mediated resistance.This is known as D-test[71].

Fluoroquinolones:

Pefloxacin,ciprofloxacin and ofloxacin have activity against Staphylococcus and can be considered for treatment. The target of Fluoroquinolones in staphylococci is topoisomerase IV DNA gyrase.A point mutation in the grl A gene ,that encodes the A subunit of topoisomerase IV leads to resistance. Thus the major limitation of fluoroquinolones is that resistance develops easily and hence have a limited role as monotherapy in serious infections[72].

Aminoglycosides:

Gentamicin,netilmicin and tobramycin are the most effective aminoglycosides against Staphylococci. But not effective as a monotherapy due to emergence of resistance. Plasmid mediated resistance develops against gentamicin[72].

Vancomycin and Teicoplanin:

These are glycopeptides active against MSSA and MRSA. Mi – Na Kim et al (2000) reported a case of Vancomycin intermediate resistance in *Staphylococcus aureus* in Korea

Mupirocin:

It is a pseudomonic acid, a natural product of Pseudomonas fluorescens.It acts by inhibiting isoleucyl-tRNA snythetase in staphylococci.It is used topically to eradicate nasal carriage.Resistance develops due to the presence of an isoleucyl-tRNA synthetase gene located on a conjugative plasmid encoding gentamycin resistance.[72]

Resistance in gram negative bacilli:

a. Extended Spectrum Beta lactamases(ESBL) [56,57]**:**

These are Bush class A plasmid mediated beta lactamases capable of hydrolyzing penicillins and monobactams and inhibited by beta lactamase inhibitors but have no detectable activity against cephamycins or carbapenems and is produced mainly by members of family Enterobacteriaceae,and also by some non fermentors.They also carry resistance for other group of antibiotics(like aminoglycosides,

fluroquinolones, cotrimoxazole etc) which narrow down the choices of antibiotics available for treatment.

Detection methods for Extended Spectrum BetaLactamases :[62]

1. Screening methods: with cefotaxime/Ceftriaxone /cefpodoxime/ceftazidime aztreonam discs by disc diffusion method.

2. CLSI phenotypic confirmatory methods: broth microdilution method/disc diffusion method.

3. Other methods: Inhibitor potentiated disc diffusion test,double disc diffusion synergy test, ESBL Epsilometer test,automated methods.

4. Molecular methods:PCR,DNA probes,PCR-RFLP,PCR-SSCP, Oligonucleotide sequencing.

AmpC production in gram negative bacilli:

Amp C beta lactamases are Bush class C beta lactamases(plasmid or chromosomal mediated), which are resistant to all beta lactamases and also to beta lactamase inhibitor combinations. They are sensitive to 4th generation cephalosporins and to carbapenems. The main Amp C producing microbes were Acinetobacter species and Klebsiella species.

Detection methodsAmpC beta lactamases :[59]

1. Screening methods: with cefoxitin disc by disc diffusion method, Cefoxitin agar method, Inhibitor based methods, AmpC disc test, Modified three dimensional test, Amp C beta lactamase Epsilometer test.

2. Molecular methods: PCR based methods

c) Metallo beta lactamases in gram negative bacilli :[60,61]

MBL These are Bush class C betalactamases capable of hydrolysing carbapenems,other beta lactams and beta lactamase inhibitors with the exception of aztreonam. They are predominantly found in Acinetobacter baumanii and Pseudomonas aeruginosa.

Detection methods for MBL:

1. Screening methods: with a carbapenemdisc (imipenem, meropenem, ertapenem etc)

2. Confirmatory methods: Imipenem –EDTA combined disc method, Imipenem EDTA double disc synergy test(DDST),EDTA disc potentiation test, HODGE test, MBL Epsilometer test.

3. Molecular methods: PCR techniques\

Treatment of secondary bacterial infection in chronic skin lesions:[10]

Antimicrobial agents that provide coverage for S. aureus as well as Cefoxitin, the carbapenems and a penicillin plus a beta lactamase inhibitor also provide cover against members of the family Enterobacteriaceae. Aminoglycosides, fourth-generation cephalosporins and quinolones should also be added to the other agents when treating these infections as per the culture and sensitivity reports.

Treatment for MRSA infection:

Strains of MRSA differ in their degree of resistance to various antibiotics. MRSA strains are usually multidrug resistant and most of them are resistant to a number of antibiotics except Glycopeptide antibiotics, but however recently MRSA with reduced susceptibility to glycopeptides has been reported.

MATERIAL AND METHODS

The present study on secondary bacterial infections associated with dermatological lesions and their antimicrobial susceptibility pattern was carried out in the Institute of Microbiology, Madras Medical College in association with the department of Dermatolgy, at the Rajiv Gandhi Government General Hospital, Chennai.

Study design & period:

Cross sectional study. One year (from September 2013 to August 2014)

Study population: Total number of 200 patients attending the department of Dermatolgy , Rajiv Gandhi Government General Hospital, Chennai were included for the study.

Ethical clearence:

Before starting the study,approval was obtained from the Institutional Ethics Committee. Informed consent was obtained from all the in-patients and out patients who satisfied the inclusion criteria.

Inclusion criteria:

- ✓ Patients older than 18 years.
- ✓ IP/OP patients with suppurative infections of skin lesions attending the Dermatology Department of RGGGH, Chennai

Exclusion criteria:

Patients less than 18 years.

Patients with non suppurative skin lesions.

Patients with sexually transmitted lesions.

Collection of data:

Data were collected from patients who satisfied the inclusion criteria, using the preformed structured questionnaire. Demographic details like name, age, sex, address, date of admission, clinical data like presenting complaints, personal history, past medical history, immunocompromised status, physical examination findings and details of clinical diagnosis were collected.

SAMPLE COLLECTION AND TRANSPORT

Samples collected:

1. Pus

2. Blood

1. Pus :

Swabs: Swabs were prepared by mounting sterile cotton wool on a stick which were introduced into test tubes and plugged. These swabs were sterilized in the hot air oven at 160°C for 1 hour.

The specimen of pus were collected aseptically with the help of three sterile swabs one for direct gram stain for detecting pus cells and microorganisms, second swab for aerobic culture and third swab for anaerobic culture. The swabs were taken from the leading edge of the wound and placed in a sterile test tube and transported to the laboratory.

2. Blood:

Under strict aseptic precautions, venepuncture site was cleaned with 70% alcohol and then with 2 % Povidone Iodine. The disinfectant was allowed to act for 1 minute and then 5ml of blood sample was collected with a sterile syringe and added into a sterile crew capped blood culture bottle containing 25 ml of sterile Brain Heart Infusion broth(BHI broth) at the bed side and transported immediately to the laboratory.

Processing of sample:

Direct Gram stain : Smear of the specimens were prepared by evenly spreading the swab on a new glass slide, air dried, heat fixed and stained using Gram staining technique. The smear was examined for the presence or absence of bacteria, their gram reaction, morphology, arrangement and pus cells.

Aerobic culture: Second swab was inoculated onto Blood agar(BA), Nutrient agar(NA) and MacConkey agar plate and were incubated at 37°C for 48 hours. If no growth was detected after 48 hours of incubation the culture was considered negative for aerobic bacterial growth.

IDENTIFICATION OF ISOLATES:-

All the isolates obtained from the pus samples were identified by standard bacteriological techniques.

IDENTIFICATION OF BETA HEMOLYTIC STREPTOCOCCI[73]:

Organisms suspected to be Beta-hemolytic streptococci from their colonial appearance on the blood agar, were identified by Gram stain and by bacitracin sensitivity.

IDENTIFICATION OF GRAM NEGATIVE BACILLI[74-76]:-

Colonies suspected to be of Gram negative bacilli from their colonial appearance on blood agar and MacConkey's agar were subjected to preliminary tests like Gram staining, hanging drop for motility, catalase and oxidase tests. Those that were Gram negative bacilli catalase positive and oxidase negative were identified as members of Enterobacteriaceae. They were identified to the species level with the

help of biochemical tests like indole, methyl red, Voges Proskauer, citrate, urease and triple sugar iron agar (TSI) tests. Those that were Gram negative bacilli catalase positive and oxidase positive, triple sugar with an alkaline slant and no change in butt, production of bright bluish-green, red or brown diffusible pigment on Muller Hinton agar were identified as pseudomonas species.

IDENTIFICATION OF STAPHYLOCOCCI COLONIES [77,79]

Colonies of Staphylococcus aureus were identified by the following characteristics.

Colony morphology on Nutrient agar:-

Colonies were large (2-4 mm diameter), circular, convex, smooth, shiny opaque and easily emulsifiable with golden yellow pigment/ white/ yellow.

Colony morphology on Blood agar:-

Colonies were 1-3 mm in diameter with a smooth glistening surface, an entire edge, smooth butryous consistency and an opaque pigmented appearance with a zone of hemolysis around them.

Colony morphology on MacConkey's agar:-

Colonies were pink and small to medium in size.

Gram staining:-

The morphology of the organisms from suspected colonies was confirmed by examining the smears by using a Gram's staining technique. Colonies showing Gram positive cocci in clusters were selected for further identification.

Carbohydrate fermentation:-

The suspected isolates were tested for aerobic and anaerobic utilization of mannitol. A loopful of bacterial culture was inoculated into peptone water containing 1% sugar and 0.2% bromothymol blue as indicator and then incubated overnight at 37°C. Change of colour of the medium from blue to yellow indicated fermentation of sugar. A small inverted durhams tube was inserted to each tube to detect gas.

Coagulase test:-

Human plasma was used for performing the coagulase test. This was obtained by centrifuging human blood, with added 0.1% EDTA, at 2000 rpm for 10 minutes.

Slide coagulase test:-

One or two staphylococcal colonies were emulsified in a drop of saline on a clean microscopic slide. If the strain was not autoagglutinable,

then undiluted plasma was added to the suspension using a Pasteur pipette. The appearance of coarse clumping visible to the naked eye within 5-10 seconds was taken as positive. Positive and negative controls were put up, to check the proper reactivity of plasma. Absence of clumping or any reaction taking more than 10 secondss were taken as negative slide coagulase test.

Tube coagulase test:-

1 in 6 dilution of the plasma was prepared in normal saline (0.85% NaCl) and 1 ml volume of it was distributed in small tubes. One - two staphylococcal colonies were inoculated and emulsified in the diluted plasma. Positive control and negative control was put up using *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* respectively. To rule out spontaneous clotting of plasma, a tube of uninoculated plasma was taken. The tubes were incubated at 37°C for upto 4 hours and were observed at 1 hour, 2 hours and 4 hours by tilting the tubes through 90°. The tubes which showed any degree of clot formation were taken as positive. The tubes in which the plasma remained wholly liquid or showed flocculent or ropy precipitate were read as negative. The negative tubes were left at room temperature overnight and re-examined in the next day.

ANTIMICROBIAL SUSCEPTIBILITY TESTING[78,80]:-

All the bacterial isolates obtained from the clinical samples of patients were tested for antimicrobial resistance pattern by using Kirby-Bauer disc diffusion method.

Antimicrobial susceptibility testing by Kirby – Bauer Disc Diffusion method:

Preparation of inoculums and Application of discs:-

1. With a sterile bacteriological wire loop 3- 5 well isolated identical colonies on an agar plate culture were touched and transferred and emulsified in 3-4ml of sterile peptone water.

2. Suspension of organism in growth medium was matched to a 0.5 McFarland standards.

3. Using a sterile cotton swab, the suspension was streaked evenly on to the surface of the cation adjusted Mueller Hinton Agar in three directions rotating the plate approximately 60 °C to ensure even distribution.

4. The surface of the inoculated agar was allowed to dry for 3 to 5 minutes with the lid in place before adding the antibiotic discs.

5. Appropriate antimicrobial discs, five discs per plate of 90mm diameter were placed on the surface of the agar using sterile forceps.

Incubation

After overnight incubation at 37°C, the diameters of zone of inhibition were measured in mm with a ruled template.

Quality control tests were done every week using the following standard ATCC control strains for testing the performance of media & drugs.

Interpretation of Zone of inhibition diameters were done according to CLSI guidelines.

ATCC control strains:

- *Staphylococcus aureus*–ATCC 25923
- *Escherichia coli*-ATCC 25922
- *Pseudomonas aeruginosa*-ATCC 27853
- *Klebsiella pneumoniae* (ESBL)-ATCC 700603

Panel of antibiotics included for testing antimicrobial sensitivity of Gram negativebacilli.

Antibiotic	Disc content	Gram negative bacilli	Diameter of Zone of inhibition in mm. Break points		
			Sensitive	**Intermediate**	**Resistant**
Amikacin	30µg		≥ 17	15-16	≤ 14
Cefotaxime	30µg	Enterobacteriaceae	≥26	23-25	≤22
		Acinetobacter	≥23	15-22	≤14
Ceftazidime	30µg	Enterobacteriaceae	≥21	18-20	≤17
		P.aeruginosa& Acinetobacter sp.	≥18	15-17	≤14
Cotrimoxazole	1.25/ 23.75µg		≥16	11-15	≤10
Ciprofloxacin	5 µg		≥21	18-20	≤17
Gentamicin	10µg		≥15	13-14	≤12
Imipenem	10µg	Enterobacteriaceae	≥23	20-22	≤19
		P.aeruginosa	≥19	16-18	≤15
		Acinetobacter	≥16	14-15	≤13
Piperacillin-Tazobactam	100µg/10 µg		**≥21**	**18-20**	**≤17**

The panel of antibiotics included in the antimicrobial sensitivity testing for Gram positive cocci were (Himedia),

Antibiotics	**Disc content**	**Zone of inhibition in mm**		
		Sensitive	**Intermediate**	**Resistance**
Amikacin	30µg	≥17	15-16	≤14
Ciprofloxacin	5µg	≥21	16-20	≤15
Cotrimoxazole	1.25/23.75µg	≥16	11-15	≤10
Chloramphenicol	30µg	≥18	13-17	≤12
Penicillin	10units	≥29	-	≤28
Erythromycin	15µg	≥23	14-22	≤13
Cefoxitin	30µg	≥22	-	≤21

METHODS FOR DETECTION OF MRSA[78,79,80,81,90]:-

1. Cefoxitin disc method:-

0.5 Mcfarland's suspension of test isolate and Staphylococcus aureus ATCC 25923 (control) was lawn cultured on cation adjusted MHA plates separately. 30 µg cefoxitin disc was placed on the surface of lawn culture of both isolates and incubated at 33–35 °C in ambient air for 16–18 hours.

Interpretation: As per CLSI guidelines

Zone of inhibition-≥22mm-MSSA

Zone of inhibition-≤21mm-MRSA

MINIMUM INHIBITORY CONCENTRATION BY EPSILOMETER TEST (E-TEST)[82]:-

All MRSA isolates were subjected to MIC estimation against oxacillin, by using E-test method (HI-MEDIA).

The E-test test strips contains antimicrobial agent with a continuous exponential gradient of antibiotics from 0.016µg to 256 µg immobilized on porous paper material and MIC values printed on both sides identically .

Procedure:

The strains were inoculated into tubes containing 2ml of peptone water. The suspension was sreaked onto the Mueller Hinton Agar with 2% Nacl to give a lawn culture. E-test strips were placed on the inoculated plates. The plates were incubated at 37°C for 24 hours and reading was taken the next day.

MIC of the drug was taken at the point where the ellipse intersects the MIC scale on the strip. Control strain ATCC Staphylococcus aureus 25923 were tested in parallel.

Interpretation: As per CLSI guidelines

MIC=≤2 μg/ml –sensitive

MIC=≥4 μg/ml –resistant

Minimum Inhibitory Concentration (MIC) for detecting Vancomycin resistance:

1. Culture media: cation adjusted Mueller Hinton broth.(pH 7.2-7.4)

2. Preparation of stock antibiotic solution: [83]

Antibiotic stock solution was prepared using the formula

$$\frac{1000}{P} \times V \times C = W$$

Where P= potency of the antibiotic in relation to the base.

(For vancomycin, P= 950/1000 mg; Himedia)

V = volume of the stock solution to be prepared (10ml)

C =final concentration of the antibiotic solution (1024µg/ml)

W = weight of the antibiotic to be dissolved in the volume V

Scheme of preparing dilution of antibiotics

Preparation of working antibiotic solution:

- Two rows of 11 sterile plugged test tubes were arranged in the racks.
- In a sterile screw capped bottle, 8ml of broth containing the concentration of antibiotic (128 µg/ml) required for the first tube in each row from appropriate stock solution (1024 µg/ml) was prepared.

- The contents of the above container were mixed thoroughly and using a sterile pipette, 1ml of the stock solution was transferred to first tubes in each row.

- Using a fresh pipette, 4ml of MH broth was added to 4ml of the stock solution , mixed well and from this concentration, 1ml was transferred to the second tube in each row

- The procedure was repeated till the 11th tube

- The first row of tubes were inoculated with test organism

- The second row of tubes were inoculated with ATCC *Staphylococcus aureus* 25923.

- 1 ml of the antibiotic free broth was placed in the last tube in each row as growth control.

- 1 ml of antibiotic solution were kept as sterility control.

- Inoculum preparation for the test and ATCC control and incubation:

- To 9.9 ml of MH broth in a sterile container , 0.1 ml of 0.5 Mcfarland turbidity matched test organism was added and mixed well.

- Using 2 ml sterile syringe, 1 ml of the above inoculum was transferred to each antibiotic containing tubes in the first row and also to the growth control tube.
- Similarly ATCC control strain inoculum was prepared and transferred to the tubes in the second row.
- These tubes were incubated at 37°C overnight.

Observation &Interpretation:

- The MIC of ATCC control strain were observed, they were within sensitive range, hence the test was considered to be valid.
- The lowest concentration of the antibiotic in which there was no visible growth was taken as the MIC of the drug for the test organism.
- **Interpretation**:

  MIC of vancomycin :

  ≤ 2µg/ml – Susceptible

  4-8µg/ml – Intermediate

  ≥16µg/ml - Resistant

MOLECULAR METHOD:

DETECTION IN OF **mecA** AND **pvl** GENES IN MRSA ISOLATES:-

Polymerase chain reaction:

All **the** MRSA isolates were subjected to PCR for the detection of methicillin resistant gene **mecA** and Staphylococcal cassette chromosome IV **pvl** GENE in the CAMRSA[102,103].

DNA extraction:

MRSA colonies (5-10) were inoculated into nutrient broth and incubated overnight at 37ºC. 1.5ml of overnight broth culture was transferred into 1.5ml of centrifuge tube and centrifuged at 10,000 rpm for 3 minutes. Supernatant was decanted; excess medium was removed by gently tapping the tube on a paper towel.

Procedure:

- The pellet obtained was suspended in 200µl of PBS.
- 180µl of Lysozyme digestion buffer and 20µl of Lysozyme were added.
- Above mixture was mixed well and incubated at 37 ºC for 15min.

- After incubation 200µl of Lysis buffer and 20µl of Proteinase K [10mg/ml] were added and incubated at 56ºC for 10min in waterbath.
- Then 300µl of Isopropanol was added and mixed well.
- The whole lysate was transferred into PureFast spin column and centrifuged at 10000rpm for 1min.
- Flow through was discarded and 500µl of Wash buffer-1 was added and centrifuged at 10000rpm for 1min.
- Flow through was discarded and 500µl of Wash buffer-2 was added and centrifuged at 10000rpm for 1min. Washing was repeated one more time.
- Flow through was discarded and the column was centrifuged for additional 2 minutes to remove any residual ethanol.
- The DNA was eluted by adding 100µl of Elution buffer and centrifuged for 1min. The eluted DNA was used as the template for PCR.

Primers [Designed by HELINI Biomolecules, Chennai]

GENE	PRIMER SEQUENCES	AMPLICON SIZE
mecA (F)	5’-GCAATCGCTAAAGAACTAAG-3’	**220bp**
(R)	5’-GGGACCAACATAACCTAATA-3’	
pvl (F)	5’-GTAAAATGTCTGGACATGATCCA– 3’	**420bp**
(R)	5’-CAACTGTATTGGATAGCAAAAGC-3’	

PCR Procedure:

Reactions were set up as follows;

Components	Quantity
HELINI 2X PCR Master Mix	10µl
Primer Mix 10pmoles/Reactions	5µl
Genomic DNA	5µl
Total volume	20µl

All the components were mixed gently and placed into PCR machine and programmed it as follows,

Cycle Number	Denaturation	Annealing	Extension
1	94ºC for 5 min	-	-
35	94ºC for 30sec	58ºC for 30sec	72ºC for 30sec
1	-	-	72º C for 5 min

Agarose gel electrophoresis:

1. 2% agarose gel was prepared with 2gm of agarose in 100ml of 1x TAE(Tris Acetate EDTA) buffer

2. To each PCR vial 8µl 6X Gel loading dye was added and mixed.

3. From each PCR vial15µl was loaded into the well.

4. Then 100bp DNA ladder and appropriate controls were loaded .

5. Electrophoresis was run at 50V till the dye reached three fourth distance and the bands were observed using UV Transilluminator.

Interpretation:

The amplified PCR products and 100bp ladder DNA molecular markers were seen as bright fluorescent bands. A 220bp corresponds to mecA, 420bp corresponds to pvl gene.

DETECTION OF ß LACTAMASE ENZYME PRODUCTION IN GRAM NEGATIVE BACILLI:

A) EXTENDED SPECTRUM ß-LACTAMASES (ESBL) DTECTION METHODS:

ESBL's are classified under Bush class A ß- lactamases which are capable of hydrolyzing penicillins – oxyiminocephalosporins and monobactams (Aztreonam) and inhibited by ß-lactamase inhibitors (clavulanic acid, sulbactum and tazobactum) but have no detectable activity against cephamycins or carbapenems (Imipenem, Meropenem).

1. **ESBL Screening method:** [85]

Isolates of gram negative bacilli showing the following resistance pattern were considered to be possible ESBL producing strains.

Antibiotic	Zone diameter for possible ESBL producing strain
Ceftazidime(30µg)	≤22mm
Cefotaxime(30µg)	≤27mm
Ceftriaxone(30µg)	≤25mm
Aztreonam(30µg)	≤27mm

2. Double Disk Diffusion Synergy Test: [83]

In this test discs of third generation cephalosporins and Augmentin [Amoxicilin and Clavulanic acid] (20µg/10µg) (Himedia) were kept 30mm apart from centre to centre on a lawn culture of 0.5 Mcfarlands test lsolate on Mueller Hinton agar (MHA) A clear extension of the edge of the inhibition zone of cephalosporin towards Augmentin disc was interpreted as positive for ESBL production.

3. Phenotypic Confirmatory Double Disk Test: (PCDDT) [84]

To 5ml of peptone water, 3-5 colonies of isolates grown on a non selective culture medium was added and incubated for 2-4 hrs at 35° C and the turbidity was matched with 0.5 Mcfarlands standard.The test was lawn cultured onto MHA plate(HiMedia,Mumbai).Ceftazidime(30µg) disc and Ceftazidime/Clavulanic acid disc (30µg/10µg) (Himedia,Mumbai) were placed on the surface of the plate and incubatedovernight at 35° C. An increase in zone diameter of ≥5mm for Ceftazidime tested in combination with Clavulanic acid versus its zone when tested alone confirmed the ESBL producing organism.

AmpC β lctamases detection methods [87]:

1)Screening method:

A lawn culture of 0.5 Mcfarland suspension of test isolate was made on MHA plate. Ceftazidime (30μg) disc were placed adjacent to cefoxitin (30μg) disc at a distance of 20 mm from each other.After overnight incubation at 35° C, isolates showing blunting of ceftazidime zone of inhibition adjacent to cefoxitin disc or showing reduced susceptibility to ceftazidime and cefoxitin were considered as screen positive.

2) AmpC disc test[88]:

On a MHA plate ,lawn culture of ATCC E.coli 25922 was prepared.On a 6mm sterile disc ,which was moistened with sterile saline,several colonies of test organism were inoculated. The inoculated disc was then placed beside a cefoxitin disc (30μg) (almost touching) on the inoculated plate. After incubation, flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disc were considered as AmpC positive isolate.

C) Metallo β lactamase (MBL) detection methods[87]:

1) Imipenem- EDTA disc method:

A 10µg imipenem disc (Hi Media) containing 750µg of EDTA solution was placed on the lawn culture of test isolate in MHA plate.After incubation,isolates which show zone diameter <14mm were considered MBL negative,whereas isolates which show zone diameter >17mm were considered MBL positive isolates.

2)Imipenem –EDTA combined disk test:

Imipenem (10 µg) and 10µg Imipenem disc containing 750µg of EDTA solution,were placed on a lawn culture of test organism on MHA plate and incubated overnight. If the increase in inhibition zone with Imipenem- EDTA disc is ≥7mm than the Imipenem disc alone, it was considered MBL positive.

Anaerobic culture: [86,89]

For anaerobic culture the specimens (i.e) pus swab, wound swabs were inoculated directly into thioglycollate broth and Robertson cooked meat broth(RCM) and transported to the laboratory.

(Thioglycollate broth was kept in boiling water bath for 10 minutes just before inoculation to drive out dissolved oxygen.)

Processing of specimen:

Specimens were inoculated onto freshly prepared and adequately dried selective anaerobic blood agar and Blood agar(BA) plates .

Inoculated plates were placed in anaerobic jar with media facing upwards. Commercially available Gas-pak was cut open at one corner and placed inside the jar and the lid of the jar was closed immediately and kept for incubation for 72 hours at 37ºC.Always a plate inoculated with Pseudomonas was put in the jar which served as a control to check maintenance of anaerobiasis.

Inoculated thioglycollate broth and Robertson cooked meat broth(RCM) was incubated at 37ºC aerobically for 48-72 hours.Then subculture was done onto BA and selective anaerobic blood agar plates and placed inside an anaerobic jar for incubation.

After appropriate period of incubation all primary as well as subculture plates were examined for evidence of growth.Colony morphology was noted. Smears were made and Gram's staining done to determine the cellular morphology.

Colonies suggestive of anaerobes was subcultured onto RCM.Aerotolerance was checked by inoculating BA plates and incubating it aerobically at 37ºC. It was examined for any growth and if

there was no growth (aerotolerance negative) it was considered as obligate anaerobe.

Blood Culture:

The patient's blood sample were inoculated into Brain Heart Infusion (BHI) Broth and incubated at 37°C aerobically and examined for turbidity at 24 and 48 hours. If turbidity or haemolysis was observed in BHI, subcultures were done onto Blood Agar and MacConkey Agar. These plates were incubated aerobically at 37°C for 24 hrs. Any growth observed was identified up to species level by colony morphology, Gram staining, catalase test, oxidase test, motility and biochemical reactions. Subcultures were done every third day for a period of 10 days and a negative report was given if no growth was observed

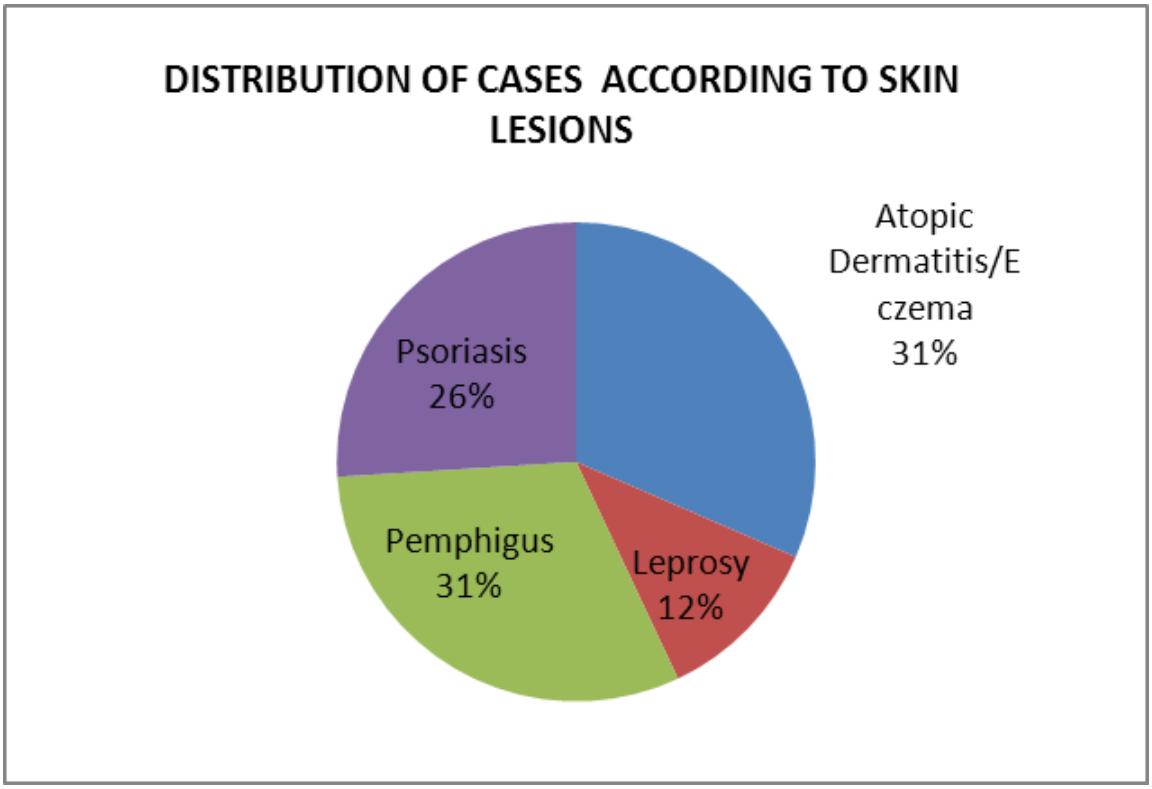
RESULTS

This cross sectional study was conducted in the Institute of Microbiology, Madras Medical College in association with the department of Dermatology, at the Rajiv Gandhi Government General Hospital, Chennai .

Total number of 200 patients with skin lesions who satisfied the inclusion criteria were included in this study from September 2013 to August 2014.

TABLE 1: DISTRIBUTION OF CASES ACCORDING TO SKIN LESIONS

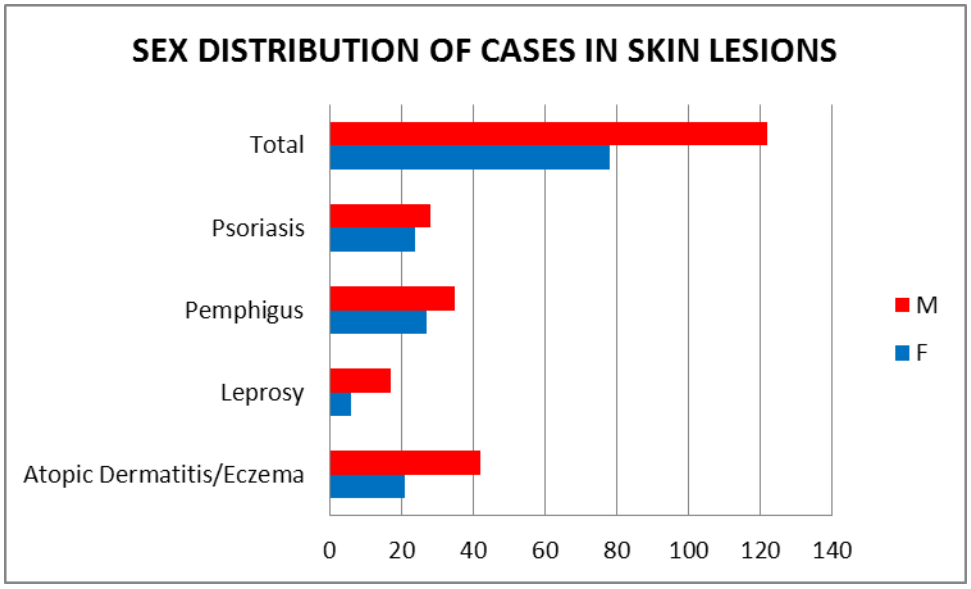
Skin Lesion	Number of cases	Percent
Atopic Dermatitis/Eczema	63	31.5
Leprosy with infected ulcer	23	11.5
Pemphigus	62	31.0
Psoriasis	52	26.0
Total	**200**	**100.0**



Out of 200 samples 63, 62,52 and 23 samples were taken respectively from patients with Atopic Dermatitis/Eczema, Pemphigus, Psoriasis and Leprosy with infected ulcer .

TABLE 2: ANALYSIS OF SEX DISTRIBUTION OF CASES IN SKIN LESIONS(n=200)

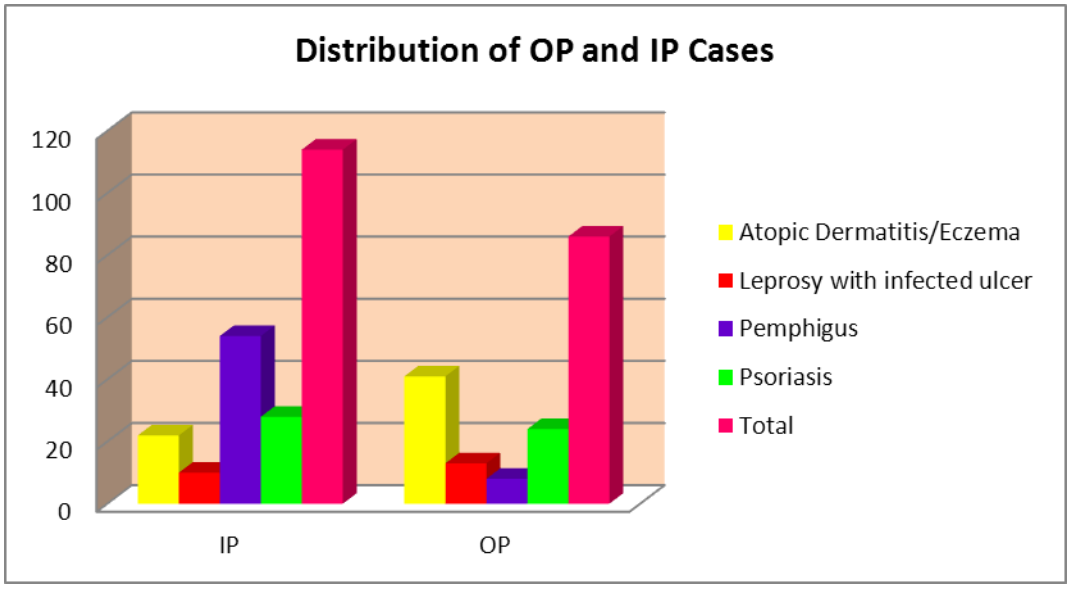
SEX	Skin Lesion				Total
	Atopic Dermatitis/Eczema	Leprosy with infected ulcer	Pemphigus	Psoriasis	
F	21(33.33%)	6(26.08%)	27(43.54%)	24(46.15%)	78(39%)
M	42(66.67%)	17(73.92%)	35(56.46%)	28(53.85%)	122(61%)
Total	**63**	**23**	**62**	**52**	**200**



Out of 200 samples 122 samples were taken from male patients and 78 from female patients.

TABLE 3 : DISTRIBUTION OF OP AND IP CASES (n=200)

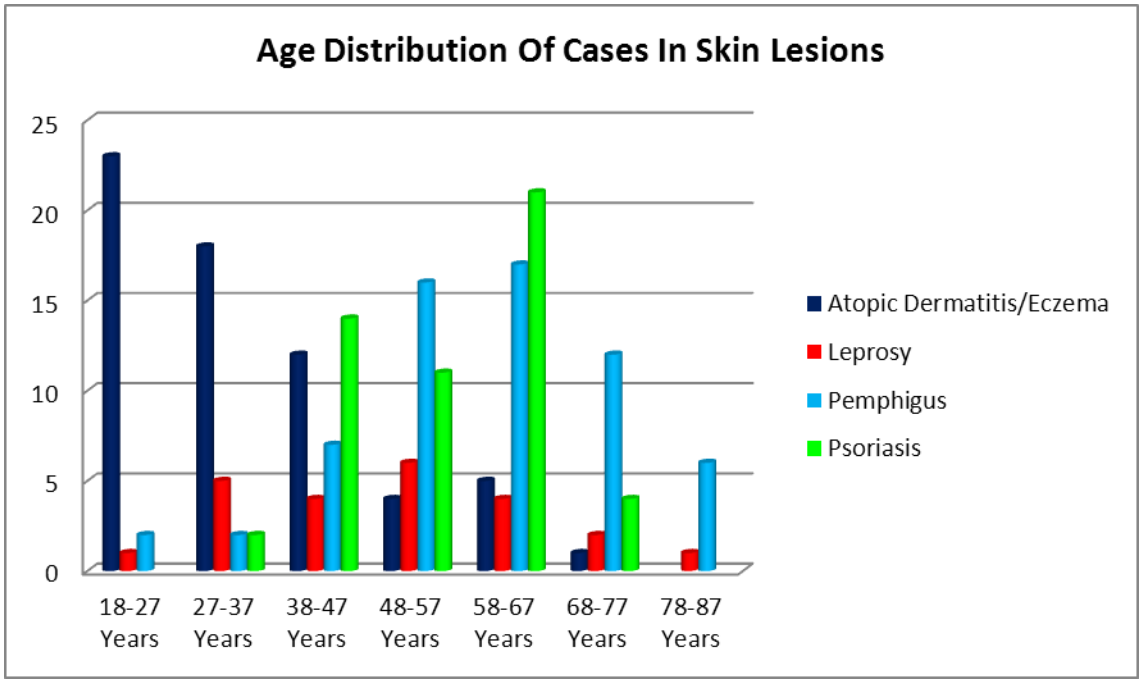
S.no	Skin Lesion	IP	Percentage	OP	Percentage
1	Atopic Dermatitis/Eczema	22	11	41	20.5
2	Leprosy with infected ulcer	10	5	13	6.5
3	Pemphigus	54	27	8	4
4	Psoriasis	28	14	24	12
	Total	**114**	**57**	**86**	**43**



Our study includes 114 IP patients (57%) and 86 OP patients (43%).

TABLE 4: ANALYSIS OF AGE DISTRIBUTION OF CASES IN SKIN LESIONS

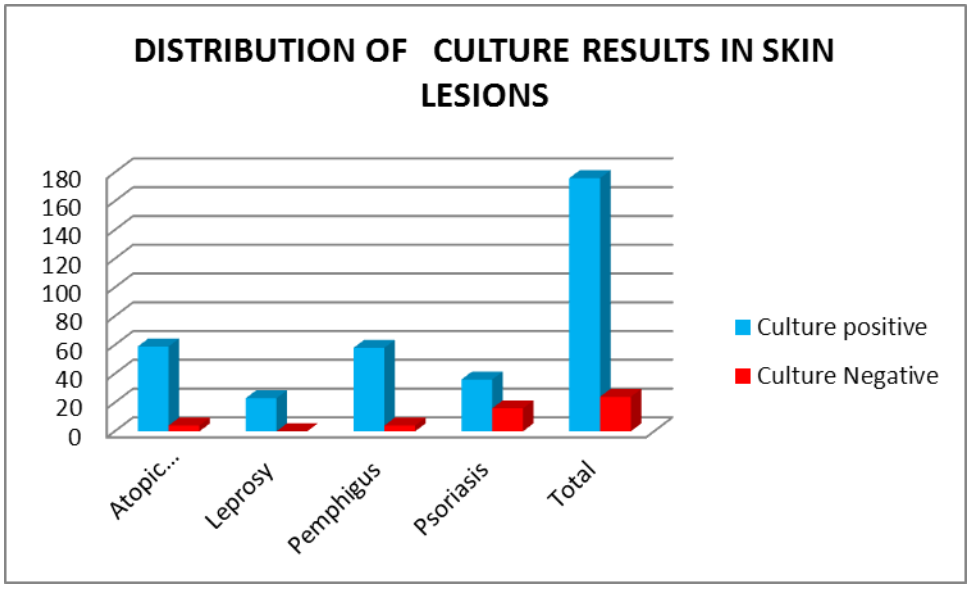
	Skin Lesion				**Total**
AGE	**Atopic Dermatitis / Eczema(n=63)**	**Leprosy with infected ulcer (n=23)**	**Pemphigus (n=62)**	**Psoriasis (n=52)**	
18-27 Years	23(36.50%)	1(4.34%)	2(3.22%)		15
27-37 Years	18(28.57%)	5(21.73%)	2(3.22%)	2(3.84%)	32
38-47 Years	1812(19.04%)	4(17.39%)	7(11.29%)	14(26.92%)	43
48-57 Years	4(6.04%)	6(26.08)	16(25.80%)	11(21.15%)	37
58-67 Years	5(7.09%)	4(17.39%)	17(27.41%)	21(40.38%)	47
68-77 Years	1(1.58%)	2(8.69%)	12(19.35%)	4(7.69%)	19
78-87 Years	-	1(4.34%)	6(9.67%)	-	7
	63	**23**	**62**	**52**	**200**



Atopic dermatitis/Eczema was more common in younger age groups, Psoriasis and pemphigus were more common in the old and middle age groups respectively.

Table: 5 DISTRIBUTION OF CULTURE RESULTS IN SKIN LESIONS

S.No	SKIN LESION	Culture positive	%	Culture Negative	%
1	Atopic Dermatitis/Eczema(n=63)	59	93.7	4	6.3
2	Leprosy with infected ulcer (n=23)	23	100	0	0
3	Pemphigus(n=62)	58	93.5	4	6.5
4	Psoriasis(n=52)	36	69.2	16	30.8
	Total	**176**	**88**	**24**	**12**



Among the various skin lesions the highest culture positivity rate was found in Leprosy with infected ulcer followed by Atopic Dermatitis / Eczema, pemphigus and psoriasis the least.

TABLE 6 :ANALYSIS OF ORGANISMS ISOLATED FROM SKIN LESIONS:(n=212)

S.No	Aerobic Gram Positive Organism	Number	%	Aerobic Gram Negative organism	Number	%	Anaerobic organism	Number	%
1	*Staphylococcus aureus*	107	50.47	*Escherichia coli*	20	9.4	*Bacteroides*	2	0.94
2	*Staphylococcus epidermidis*	10	4.71	*Klebsiella oxytoca*	12	5.6	*Peptostreptococci*	4	1.88
3	*Streptococcus pyogenes*	10	4.71	*Klebsiella pneumoniae*	6	2.83			
4				*Pseudomonas aeruginosa*	29	13.68			
5				*Proteus vulgaris*	10	4.71			
6				*Proteus mirabilis*	2	0.94			
	Total	**127**	**59.9**		**79**	**37.27**		**6**	**2.82**

Aerobic gram positive organisms accounts for 59.9% followed by Aerobic gram negative 37.27% and anaerobic organisms 2.83%. *Staphylococcus aureus* was the most commonly isolated organism in this study (50.47%), followed by,enterobacteriacea(23.59%), *Pseudomonas aeroginosa* (13.68%) and anaerobic organisms (2.83%).

TABLE 7: ANALYSIS OF ORGANISMS ISOLATED FROM IP AND OP CASES:

S.no	IP		OP		Total
	Organism	**Nos**	**Organism**	**Nos**	
1	*Staphylococcus aureus*	60 (46.8%)	*Staphylococcus aureus*	47 (55.95%)	107
2	*Pseudomonas aeruginosa*	18(14.06 %)	*Pseudomonas aeruginosa*	11(13.09 %)	29
3	*Escherichia coli*	14(10.09 %)	*Escherichia coli*	6(7.14%)	20
4	*Staphylococcus epidermidis*	6(4%)	*Staphylococcus epidermidis*	4(4.76%)	10
5	*Streptococcus pyogenes*	4(3.12%)	*Streptococcus pyogenes*	6(7.14%)	10
6	*Klebsiella oxytoca*	9(7%)	*Klebsiella oxytoca*	3(3.57%)	12
7	*Klebsiella pneumoniae*	5(3.9%)	*Klebsiella pneumonia*	1(1.19%)	6
8	*Peptostreptococci*	3(2%)	*Peptostreptococci*	1(1.19%)	4
9	*Proteus vulgaris*	6(4%)	*Proteus vulgaris*	4(4.76%)	10
10	*Proteus mirabilis*	1(0.78%)	*Proteus mirabilis*	1(1.19%)	2
11	*Bacteroides*	2(1.56%)	*Bacteroides*	0	2
	Total	**128 (60.37%)**		**84 (39.62%)**	**212**

Staphylococcus aureus was the most commonly isolated organisms in both IP and OP cases.Enteric gram negative bacilli were more in IP cases than in OP cases.

TABLE 8: ANALYSIS OF ORGANISMS ISOLATED FROM DIFFERENT ANATOMICAL SITES

S.no.	ORGANISM	SITE OF LESION			
		UPPER LIMB	LOWER LIMB	TRUNK	HEAD&NECK
1	*Staphylococcus aureus*	45	41	18	3
2	*Pseudomonas aeruginosa*	4	19	2	4
3	*Escherichia coli*	4	12	3	1
4	*Staphylococcus epidermidis*	4	0	4	2
5	*Streptococcus pyogenes*	6	2	2	0
6	*Klebsiella oxytoca*	4	6	0	2
7	*Klebsiella pneumoniae*	0	2	4	0
8	*Peptostreptococci*	2	0	0	2
9	*Proteus vulgaris*	2	8	0	0
10	*Proteus mirabilis*	0	0	2	0
11	*Bacteroides*	0	2	0	0
Total		**71**	**92**	**35**	**14**

Escherichia coli was more commonly isolated from lower limbs than any other sites.

Anaerobic *Peptostreptococci* were isolated from head and neck and *Bacteroides* from lower limbs.

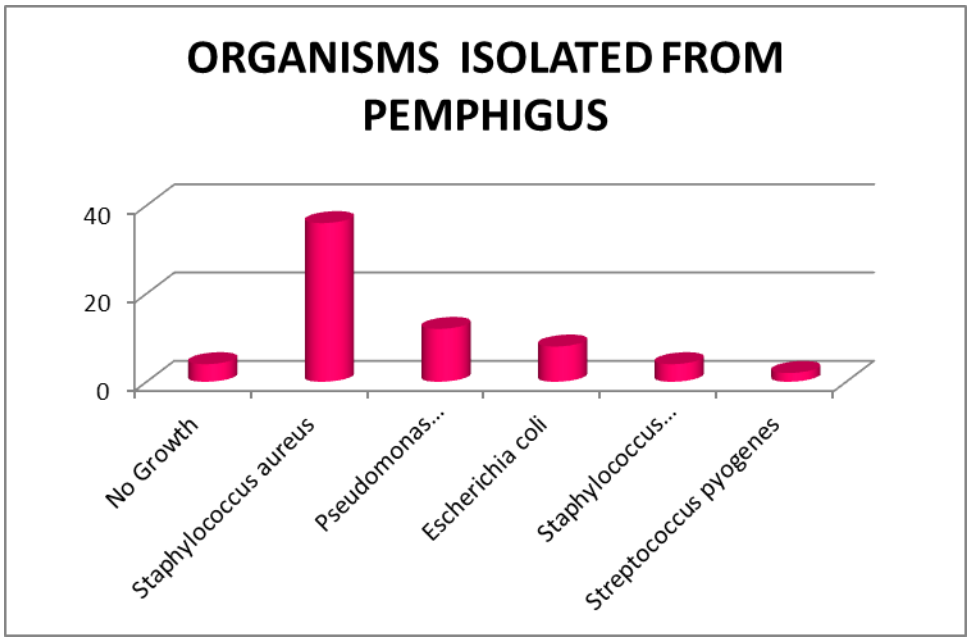
TABLE 9: DISTRIBUTION OF BACTERIAL ISOLATES IN MIXED INFECTIONS IN SKIN LESIONS

S.no	Lesion	Organism 1	Organism 2	Number of mixed isolates
1	Atopic Dermatitis / Eczema (n=63)	*Staphylococcus aureus*	*Peptostreptococci*	2
		Staphylococcus aureus	*Klebsiella oxytoca*	2
		Staphylococcus aureus	*Escherichia coli*	2
		Staphylococcus aureus	*Bacteroids*	2
Total				**8 (12.69%)**
2	Leprosy with infected ulcer (n=23)	*Klebsiella oxytoca*	*Escherichia coli*	2
		Staphylococcus aureus	*Pseudomonas*	2
Total				**4 (17.39%)**
3.	Pemphigus (n=62)	*Pseudomonas*	*Peptostreptococci*	2
		Staphylococcus aureus	*Klebsiella pneumonia*	1
		Staphylococcus aureus	*Klebsiella oxytoca*	1
		Staphylococcus aureus	*Escherichia coli*	2
		Staphylococcus aureus	*Pseudomonas*	6
		Staphylococcus aureus	*Streptococcus pyogenes*	2
		CONS	*Pseudomonas*	2
Total				**16 (25.80%)**
4.	Psoriasis (n=52)	*Staphylococcus aureus*	*Escherichia coli*	2
		Staphylococcus aureus	*Proteus vulgaris*	4
		Staphylococcus epidermidis	*Proteus mirabilis*	2
Total				**8 (15.38%)**

Mixed infections were found highest in Pemphigus(25.80%) followed by Leprosy with infected ulcer ,Psoriasis and Atopic Dermatitis/Eczema.

TABLE 10: ANALYSIS OF ORGANISMS ISOLATED FROM PEMPHIGUS (n=74)

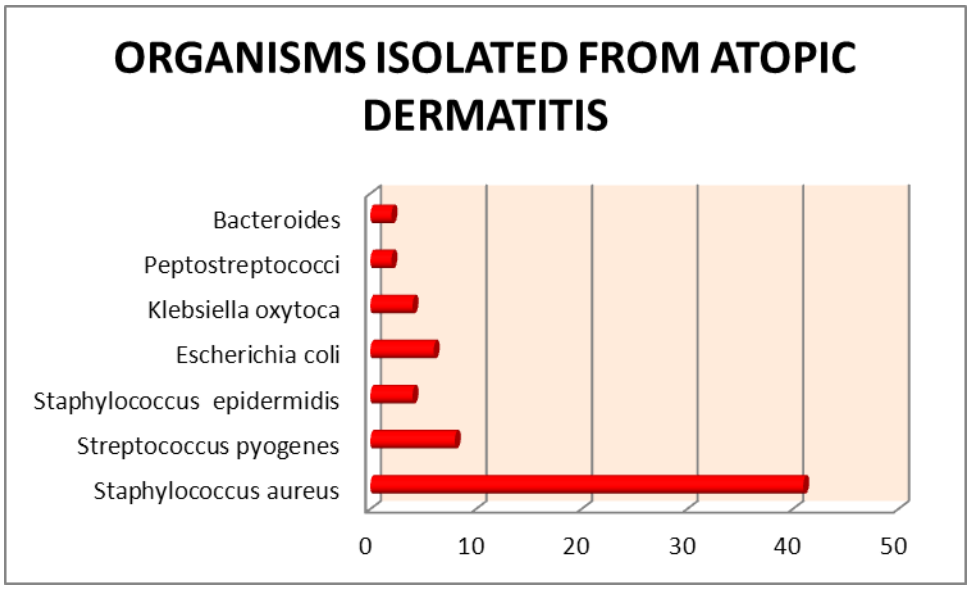
S.no	ORGANISM	No	Percentage
1.	*Staphylococcus aureus*	36	48.64
2.	*Staphylococcus epidermidis*	4	5.4
3.	*Streptococcus pyogenes*	2	2.7
4.	*Pseudomonas aeruginosa*	12	16.21
5.	*Escherichia coli*	8	10.81
6.	*Klebsiella oxytoca*	4	5.4
7.	*Klebsiella pneumoniae*	6	8.10
8.	*Peptostreptococci*	2	2.70
Total		**74**	



Staphylococcus aureus(48.64%) were the most common organism in pemphigus followed by *Pseudomonas aeruginosa* ,Enterobacteriaceae , *Streptococcus pyogenes* and Anaerobic organisms.

TABLE 11: ANALYSIS OF ORGANISMS ISOLATED FROM ATOPIC DERMATITIS (n=67)

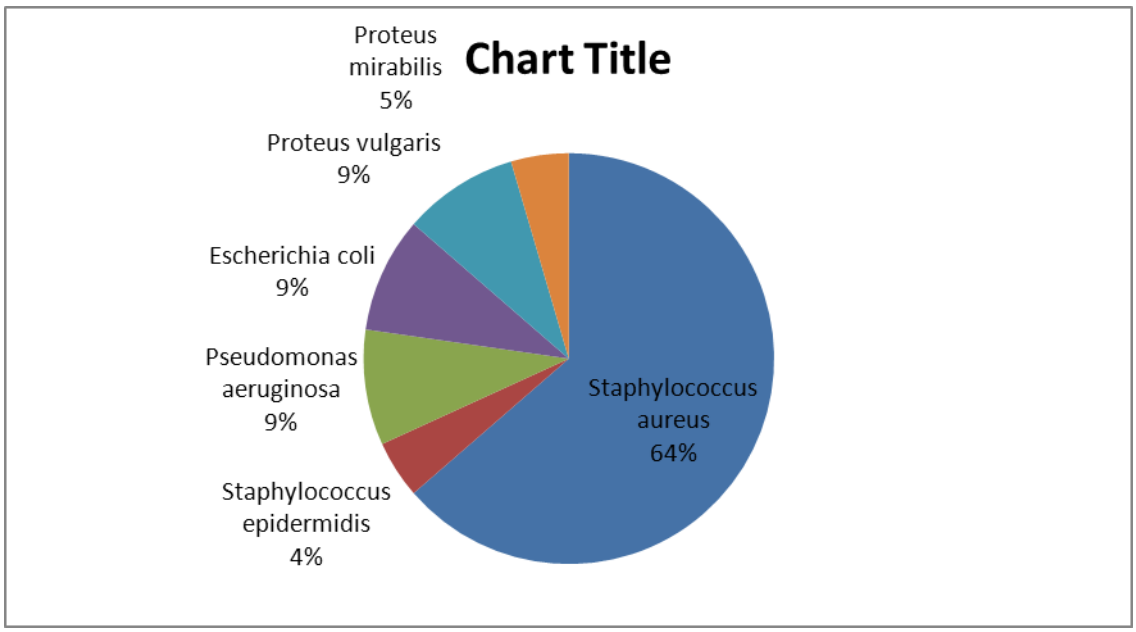
S.No	ORGANISM	No	%
1.	*Staphylococcus aureus*	41	61.19
2.	*Streptococcus pyogenes*	8	11.94
3.	*Staphylococcus epidermidis*	4	5.97
4.	*Escherichia coli*	6	8.95
5.	*Klebsiella oxytoca*	4	5.97
6.	*Peptostreptococci*	2	2.98
7.	*Bacteroides*	2	2.98
Total		**67**	



61.19% of isolates were *Staphylococcus aureus* followed by *Streptococcus pyogenes*,Enterobacteriacea and anaerobic organisms.

TABLE 12: ANALYSIS OF ORGANISMS ISOLATED FROM PSORIASIS(n=44)

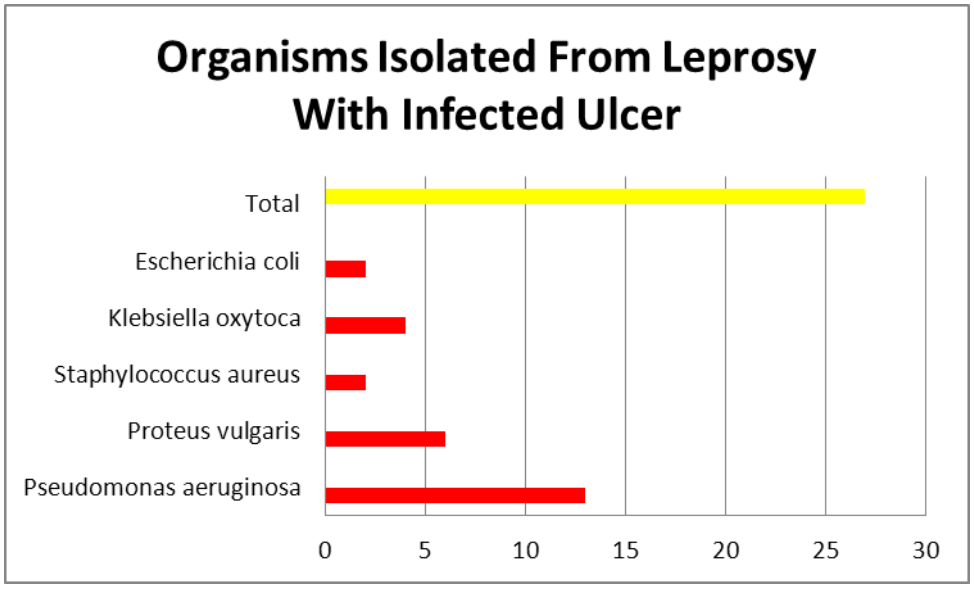
S.No	ORGANISM	No	%
1.	*Staphylococcus aureus*	28	63.63
2	*Staphylococcus epidermidis*	2	4.54
3.	*Pseudomonas aeruginosa*	4	(9.09
4.	*Escherichia coli*	4	9.09
5.	*Proteus vulgaris*	4	9.09
6.	*Proteus mirabilis*	2	4.54
Total		**44**	



Most common isolate in Psoriasis was *Staphylococcus aureus* (63.3%) followed by Enterobacteriacea and Staphylococcus epidermidis.

TABLE 13: ANALYSIS OF ORGANISMS ISOLATED FROM LEPROSY WITH INFECTED ULCER

S.No	ORGANISM	No	%
1.	*Pseudomonas aeruginosa*	13	48.1
2.	*Proteus vulgaris*	6	22.2
3.	*Staphylococcus aureus*	2	7.4
4.	*Klebsiella oxytoca*	4	14.8
5.	*Escherichia coli*	2	7.4
Total		**27**	



Pseudomonas aeruginosa (48%) was the most common organism in Leprosy with infected ulcer followed by *Proteus vulgaris*(22%).

TABLE 14 : ANALYSIS OF BLOOD CULTURE RESULTS:

S.No	Skin lesion	BLOOD CULTURE			
		POSITIVE	ORGANISM	NEGATIVE	TOTAL
1	Pemphigus(n=62)	2(3.22%)	MRSA	9	11
2	Psoriasis(n=52)	0	-	6	6

Blood culture were taken for the patients with clinical signs of sepsis like fever.

All of them were Inpatients.

Two cases of pemphigus patients were positive for culture. (3.22%)

Methicillin resistant *Staphylococcus aureus* was isolated from both the patients.

TABLE 15 :ANTIBIOTIC SENSITIVITY PATTERN OF GRAM POSITIVE ORGANISMS

S.no	ORGANISM	Total no	Amikacin		Ciprofloxacin		Cotrimoxazole		Chloramphenicol		Penicillin		Erythromycin	
			S	%	S	%	S	%	S	%	S	%	S	%
1	*Staphylococcus aureus*	107	84	78.5	52	48.6	39	36.4	-	-	39	36.4	41	38.3
2	*Staphylococcus epidermidis*	10	9	90	6	60	4	40	-	-	6	60	9	90
3	*Streptococcus Pyogenes*	10	8	80	8	80	0		7	70	10	100	7	70

All gram positive organisms were highly sensitive to amikacin and least sensitive to penicillin and cotrimoxazole.

TABLE 16 : ANTIBIOTIC SENSITIVITY PATTERN OF GRAM NEGATIVE BACILLI

S.NO	Antibiotic	*Escherichia coli* (n = 20)		*Klebsiella oxytoca* (n = 12)		*Klebsiella pneumonia* (n = 6)		*Pseudomonas aeruginosa* (n = 29)		*Proteus vulgaris* (n = 10)		*Proteus mirabilis* (n = 2)	
		S	%	S	%	S	%	S	%	S	%	S	%
1	Amikacin	17	85	7	58.3	4	66.7	22	75.9	7	70	1	50
2	Cefotaxime	10	50	1	0.8	4	66.7	17	58.6	8	80	0	0
3	Ceftazidime	10	50	1	0.8	4	66.7	17	58.6	8	80	0	0
4	Cotrimoxazole	7	35	1	0.8	1	16.7	0	0	4	40	0	0
4	Ciprofloxacin	7	35	0	0	2	33.3	12	41.4	0	0	0	0
6	Gentamicin	14	70	2	1.6	2	33.3	13	44.8	2	20	0	0
7	Imipenem	20	100	12	100	6	100	29	100	10	100	2	100
8	Piperacillin/ Tazobactum	20	100	12	100	6	100	29	100	10	100	2	100

S-sensitive

All gram negative bacilli were 100% sensitive to imipenem and piperacillin-tazobactum.

All gram negative isolates showed higher rate of sensitivity to amikacin and least to ciprofloxacin

TABLE 17 :RESISTANT PATTERN IN GRAM NEGATIVE ORGANISM

S.NO	ORGANISM	TOTAL NOS	ESBL	DDST	PCDDT	PERCENTAGE
1	*Escherichia coli*	20	10	10	10	50
2	*Klebsiella oxytoca*	12	10	10	10	83.33
3	*Klebsiella pneumoniae*	6	3	3	3	50
4	*Proteus mirabilis*	2	2	2	2	100
	Total	**40**	**25**	**25**	**25**	**62.5**

DDST – Double disk diffusion synergy test

PCDDT – Phenotypic Confirmatory disk diffusion test

ESBL producers were 62.5%. All the ESBL producers were sensitive to Imipenem.

There was no Amp C ,and MBL producers in this study.

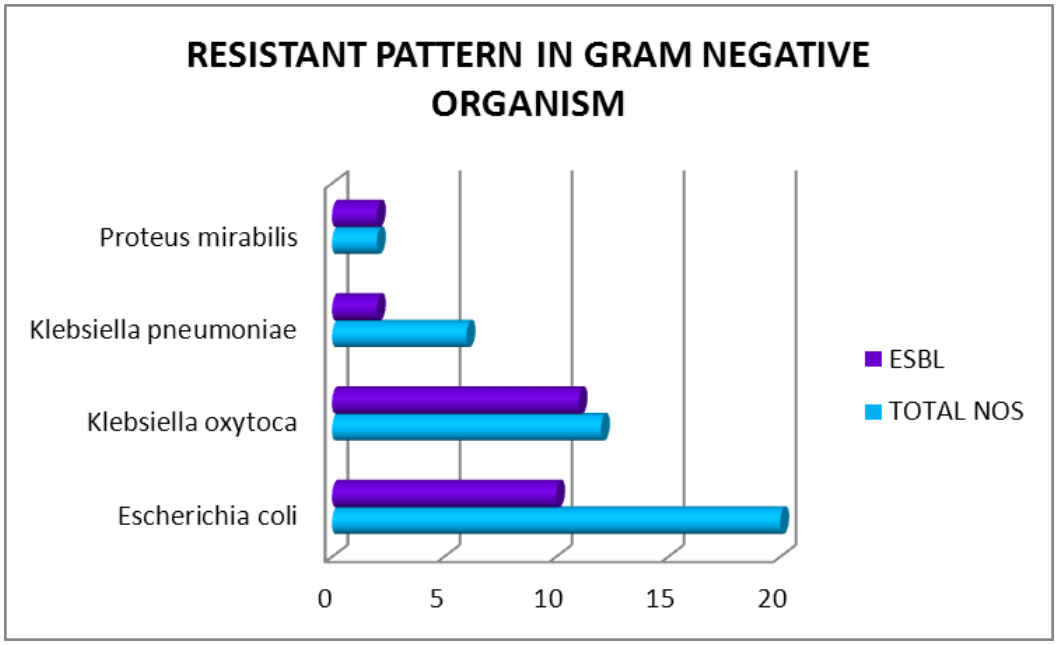
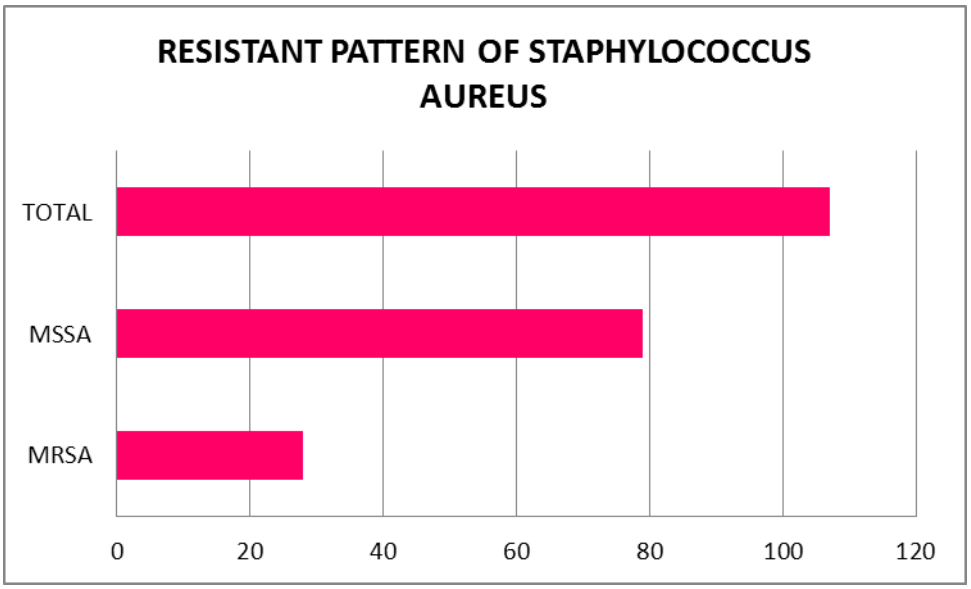


TABLE 18: RESISTANT PATTERN OF STAPHYLOCOCCUS AUREUS

S.NO	ORGANISM		NOS	PERCENTAGE
1	MSSA		69	64.48
2.	MRSA	CEFOXITIN DISC DIFFUSION	38	35.52
		OXACILLIN E-TEST	38	35.52
	TOTAL		**107**	

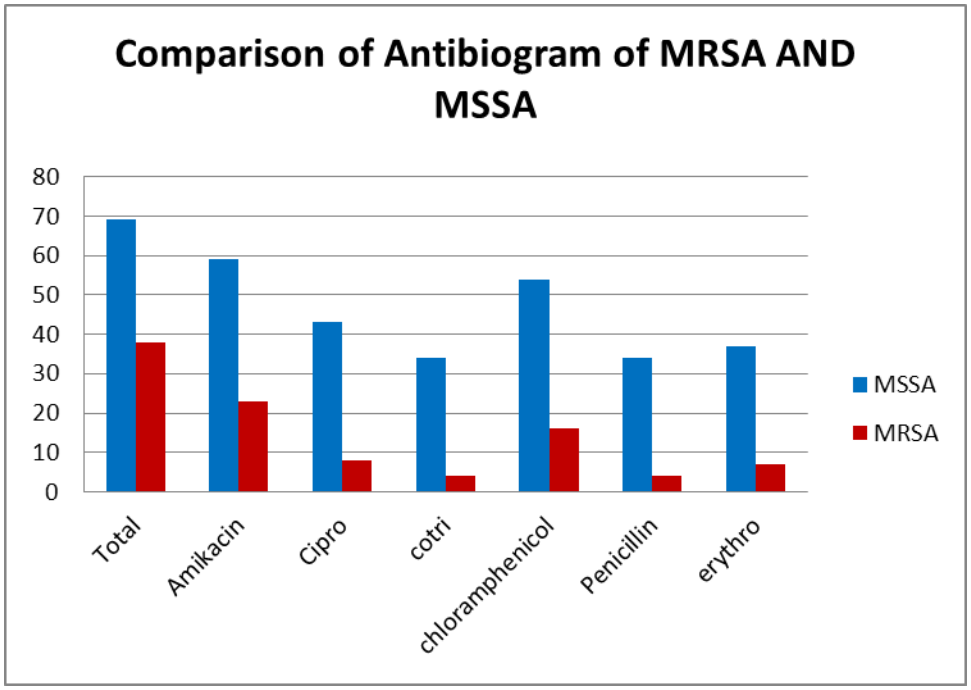


Out of 107 Staphylococcus aureus 69(64%) were MSSAand 38(36%) were MRSA.

TABLE 19: Comparison of Antibiogram of MRSA AND MSSA

S.No	Staphylococcus aureus	Total	Amikacin		Ciprofloxacin		Cotrimoxazole		Penicillin		Erythromycin	
			S	%	S	%	S	%	S	%	S	%
1	MSSA	69	59	85.50	43	62.32	34	49.27	34	49.27	37	53.6
2	MRSA	38	23	60.52	8	21.1	8	21.05	4	10.52	7	18.42
3	P-value(by-Pearson Chi-Square test)			0.001		0.001		0.001		0.001	0.001	
				S		S		S		S	S	

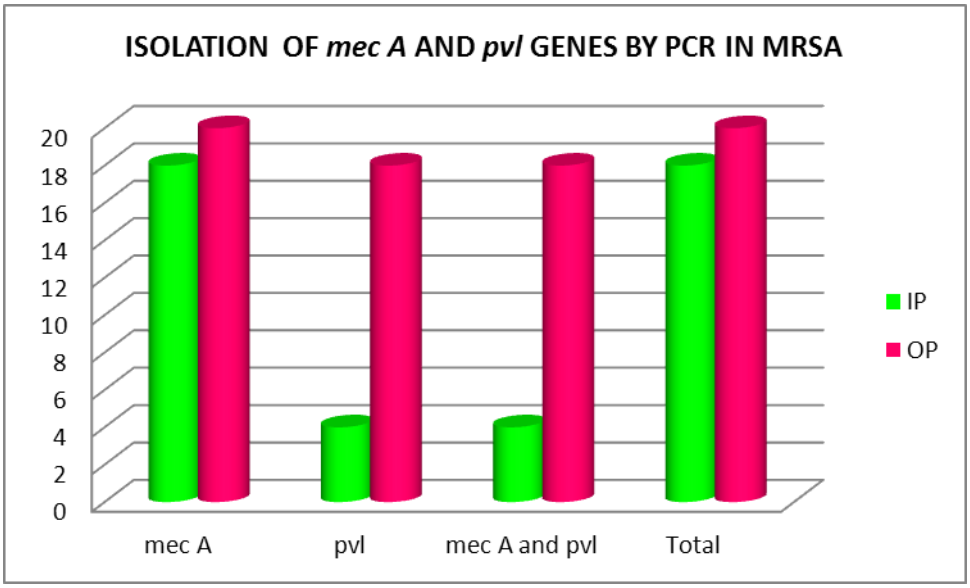
S-Significant



MRSA strains were more resistant to all antimicrobial agents than MSSA and it was statistically significant.

TABLE 20:ISOLATION OF mec A AND pvl GENES BY PCR IN MRSA

S.No.	GENE	IP(n=18)	OP(n=20)
1	mec A	18(100%)	20(100%)
2	Pvl	4(22.22%)	18(90%)
3	mec A and pvl	4(22.22%)	18(90%)



All MRSA were positive for mecA gene.

MRSA from OP patients(CA MRSA) showed higher positivity for Pvl gene 90% and only 22% of MRSA from IP patients(HA MRSA) were positive for Pvl gene

TABLE21:INTERPRETATION OF MIC OF VANCOMYCIN FOR METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS BY MACROBROTH DILUTION METHOD

SKIN LESION	no OF MRSA	MIC value	Interpretation
Pemphigus	17	≤ 2µg/ml	Sensitive
Atopic dermatitis/eczema	13	≤ 2µg/ml	Sensitive
Psoriasis	8	≤ 2µg/ml	Sensitive

All the Methicillin resistant *Staphylococcus aureus* were sensitive to vancomycin.

DISCUSSION

This cross sectional study was conducted in the Institute of Microbiology, Madras Medical College in association with the department of Dermatology , at the Rajiv Gandhi Government General Hospital, Chennai .

Total number of 200 patients with skin lesions who satisfied the inclusion criteria were included in this study.

In this study out of 200 samples, 63(31.5%) samples were taken from Atopic Dermatitis/Eczema cases, 62(31%) from Pemphigus , 52(26%) Psoriasis and 23 (11.5%) samples were taken from Leprosy with infected ulcer cases .(TABLE:1)

Out of 200 samples 122 samples were taken from male patients and 78 samples were taken from female patients. (TABLE 2).In cases of leprosy with infected ulcer male cases were common. This was similar to the findings of Tsehayneshlema et al which explained this predominance, as true difference between males and females and is not because of the under diagnosis in women, due to delayed presentation of female patients, which results in high deformity . In all skin lesions male cases were more than the female cases.

114 (57%) cases were from In patients and 86(43%) cases were from out patients in this study(TABLE 3)

Atopic Dermatitis cases were more common in the younger age groups in this study. (TABLE 4) J.Q. Gong et al 2006 and Wollenberg A Kraft S et al 2000 reported similar age distribution due to the development of tolerance to the allergens as age advances[25,26,27].

Though Psoriasis can occur at any age ,it was common in the age group of 30 -60 years in this study which was similar to the studies by Shehab A. Lafi et al 2010 [27] and Abdullah, M.; et al. (2007)[1]. These findings disagree with the findings of Hwerta, C. et al. 2007 [92] and Zieve, d. et al.2008 in which higher rate of Psoriasis was reported in the age group of 18-40 years where most of the cases were Guttate psoriasis that is more common in younger age group . This discrepancy might be due to difference in the type of psoriasis cases included in this study most of which were Psoriasis vulgaris that are common in the older age group(40-60 years).

Pemphigus cases were common between the age group of 40-70 years in this study and Leprosy with infected ulcer shows no significant age distribution.

In this study out of 200 cases organisms were isolated from 176 cases (88%) and in 24 cases(12%) culture were negative(TABLE5).

The positivity rates differs among the skin lesions. In Atopic Dermatitis/eczema 93.7% cases were culture positive which was similar to the study by Lübbe J 2003[26].

69.2% of Psoriasis cases were culture positive in this study which was similar to the study by Itzhak brook et al 2002[10], but differs from Shehab A. Lafi1 et al 2010 [27], which showed a higher rate of isolation(88%).

Endogenous antimicrobial peptides such as cathelicidins and β defensins are over expressed in Psoriatic skin which was the reason for the lower rate of secondary infection than Atopic dermatitis in which these peptides are under expressed [93].

In Pemphigus 93.5% of cases showed positive cultures which was in accordance with the studies by Nousari HC et al 1999 and various other studies[32,33,34,36].

In Leprosy with infected ulcer all the patients showed positive culture which was similar to Tsehayneshlema et al 2012 [44] [TABLE 6]

Total number of organisms isolated in this study were 212.

127(59.9%) were aerobic gram positive organisms in which *Staphylococcus aureus* were 107(50.47%) followed by *Staphylococcus epidermidis*10(4.71%) and *Streptococcus pyogenes*10(4.71%).

Similarly, Brook I et al 2002 and Marwa Abdallah 2007[3,1] mentioned that Staphylococcus aureus was the commonest organism causing secondary infection of skin lesions and represented , 43.5% and 61.8% of all positive cultures respectively. This might be related to the inhibitory effect of serum exuding from damaged skin on linolenic acid. Linolenic acid is an essential free fatty acid normally present on intact skin, which is responsible for inhibition of Staphylococcus aureus colonization [95].

79(37.26%) were aerobic gram negative organisms in which enteric gram negative bacilli were 50(23.59%) followed by *Pseudomonas aeruginosa*29(13.67%). This was similar to the studies by Brook I et al 2002 and Marwa Abdallah et al 2007[3,1] where enteric gram negative bacilli was in the range between 17.3to24.7%

6(2.83%) were anaerobic organisms with 4(1.88%) being Gram positive cocci and 2(0.94%) being Gram negative rods. This finding was similar to the findings of Abdallah et al 2007, Brook I et al 2002 , [1,3] which reported (1.7-7.1%) . (TABLE 7)

Out of 212 isolates 128(60.37%) were from IP cases and 84(39.62%) were from OP cases. *Staphylococcus aureus* was the most common organism isolated from both the cases and there was no significant difference between the isolation rate of all organisms ,except for the gram negative enteric organisms which were significantly more in inpatients .This was similar to the findings in the study by Marwa Abdallah etal 2007[1].(TABLE 8).

In this study *Staphylococcus aureus*, was the most prevalent aerobe, isolated from all body sites. Similar findings were noted in the study by Marwa Abdallah et al 2007 [1] suggesting that this organism may induce purulent superinfection as well as enhance the inflammatory process by superantigen-mediated T-cell activation [95] .

Enteric gram-negative bacilli and *Bacteroides spp*. were found most common in the leg lesions. The probable sources of these organisms might be from the rectum and vagina, where they normally reside [97]. *Streptococcus pyogenes* were most commonly found in lesions of the upper limb. These organisms probably reached these sites from the oral cavity, where they were part of the normal flora [11] (TABLE 9).

Mixed infections in Pemphigus were 25.80% in this study followed by Leprosy with infected ulcer (17.39%), Psoriasis(15.38%) and Atopic Dermatitis (12.69%) respectively.

Marwa Abdallah 2007[1] reported similarly between 15 to 30% mixed infections in various skin lesions. The higher rate of polymicrobial infections in Pemphigus and Psoriasis lesions were attributed to the potential for bacterial synergy and immunosuppressive drugs that are indispensible in the treatment of these patients[10].This findings disagree with the findings from the studies by Brook I et al 2002 and various other studies which reported higher rates of mixed infections (30 to50%) [1,4,13].

Brook I et al and several studies have documented the synergestic effect of mixtures of aerobic and anaerobic bacteria in skin and soft tissue infections . Various hypotheses have been proposed to explain such microbial synergy. It may be the result of loss of protection from phagocytosis and intracellular killing , or lowering of oxidation-reduction potentials in host tissue [95,96] . (TABLE10)

Staphylococcus aureus(48.64%) were the most common organism in pemphigus followed by *Pseudomonas aeruginosa* ,Enterobacteriaceae , *Streptococcus pyogenes* and anaerobic organisms in this study. This was similar to the findings of Marwa Abdallah et al 2007[1] and Nafiseh Esmaili et al 2013[1,13] . Studies by Itzhak brook et al 2002 and various

studies showed different pattern of infection in which Enterobacteriaceae was the second most common cause followed by *Staphylococcus aureus*[3,33,35] .(TABLE 11)

In this study61.19% of isolates from Atopic Dermatitis/Eczema were Staphylococcus aureus followed by Streptococcus pyogenes ,

Enterobacteriacea and anaerobic organisms. (TABLE12)This was similar to the studies by Marwa Abdallah et al 2007 and Itzhak brook et al 2002[1,3] which reported between60-90%. This finding might be due to the higher colonisation of *Staphylococcus aureus* in atopic dermatitis patients that might play a role in its pathogenesis.

In this study most common isolate in Psoriasis was *Staphylococcus aureus* (63.63%) followed by Enterobacteriacea and *Staphylococcus epidermidis.*

Staphylococcus aureus was predominant isolated bacteria from psoriatic patient lesions,(TABLE13) this was in agreement with Gudionsson, E. J. et al. 2003 and various other studies[98,1,3,27] .

Staphylococcus aureus was the commonest organism causing secondary infections of skin lesions, this might be attributed to the antiphagocytic effect of protein A of this organism, as well as the inhibitory effect of serum exuding from denuded skin on linolenic acid ,

which is an essential fatty acid normally present on the intact skin that helps in inhibiting *Staphylococcus aureus* colonization[27]. Isolation of opportunistic pathogens from psoriatic lesions , *Staphylococcus epidermidis* was due to the reduced local defense factors in the lesion due to local and systemic immunosuppressant drugs like cytotoxic drugs and corticosteroids used for the treatment of psoriasis[27].

In this study *Pseudomonas aeruginosa* (48%) was the most common organism isolated from Leprosy with infected ulcer followed by *Proteus vulgaris*(22%),*Klebsiella oxytoca, Staphylococcus aureus* and *Escherichia coli*.(TABLE 14)

This coincides with Srinivasan H et al 2004 and various other studies from India which showed the predominant isolate was *Pseudomonas aeruginosa*[40,41,42,43]. This findings disagree with the study by Tsehayneshlema et al 2012 [44] in Ethiopia which showed Proteus as the predominant organism isolated.

Blood cultures were done for the patient with clinical signs of sepsis like fever. It was done for17 In patients [11 from Pemphigus(1.77%) and 6 from Psoriasis(1.15%)] .MRSA was isolated from two cases of Pemphigus. (TABLE 15)

This finding was different from the study by Ljubojevi´c et al on 159 patients done for a period of 19 years revealed that incidence of sepsis was about5.66%.This low rate of sepsis in our study might be due to the small number of patients and the shorter period of our study. The severe complications were due to the high doses of corticosteroids and immunosuppressive therapy given to these patients.[12]

A. Razzaque Ahmed et al [34] stated that Staphylococcus aureus was the commonest organism isolated from secondarily infected lesions of pemphigus and the septicemia resulting from it was the most important cause of death. Hence, cautious observation and antiseptic care for pemphigus lesions, along with a judicious use of steroids is mandatory.

All gram positive organisms were highly sensitive to amikacin (78-90%) and least sensitive to cotrimoxazole and penicillin(36.4%).(TABLE 16)

This finding differs from other studies by Marwa Abdallah et al 2007 and Itzhak brook et al 2002[1,3] where they reported higher sensitivity rate for cotrimoxazole(60-78%) , which might be due to the use of different antibiotic regimen in different settings.

In this study all Gram negative organisms were 100% sensitive to imipenem and piperacillin-tazobactum, and higher rate of sensitivity to

amikacin and least sensitive to quinolones. This is similar to the findings of Marwa Abdallah et al 2007[1] (TABLE 17)

ESBL producers in this study were 62.5%.[TABLE18].This was different from that of the study by Marwa Abdallah et al 2007and various studies [1,13,36] which showed lower rates between 10-40%.

All the organisms were sensitive to Imipenem and there were no AmpC or MBL producers in this study which was similar to that by S. Ljubojevi´c et al and Marwa Abdallah et al 2007[12,1].

36%of Staphylococcus aureus were Methicillin resistant in this study. (TABLE19)This correlates with the reports of various studies in India which ranges between20-40%. In 1996, Pulimood from Vellore reported 24%[50].The following year Udaya Shankar from Pondicherry reported 20%.In 2006, Rajaduraipandi reported 37.9% from Coimbatore.[50,51,52] A study conducted by INSAR group ,showed that the prevalence of MRSA in our country is about 40 %.[63]

All 38 isolates of MRSA were detected by cefoxitin disc diffusion and oxacillin MIC by E-test methods. This finding was similar to the studies by Mathew AA et al2010 and Anand KB et al 2009[105,106] which showed 100% sensitivity and specificity in detecting mecA mediated MRSA by cefoxitin disc diffusion method.

When comparing the antimicrobial susceptibility pattern of MSSA and MRSA in this study MRSA isolates were found to be more resistant to the commonly used antibiotics than MSSA. Significant difference was observed in case of erythromycin, ciprofloxacin, cotrimoxazole, penicillin and amikacin.[P<0.05]. (TABLE:21)This was similar to the study conducted by INSAR group[63]

All the MRSA isolates in this study were subjected to conventional PCR for the detection of methicillin resistant gene mecA and pvl toxin gene.

Out of 18 MRSA isolated from IP patients 18(100%) were positive for mecA gene, 4 (22%)were positive for pvl gene and 4(22%) were positive for both . (TABLE21) Out of 20 MRSA isolated from OP patients,20(100%) were positive for mecA and 18(90%) were positive for pvl gene.

Similar to our study Binh An Diep et al 2004 and various studies reported prevalence of pvl gene between 70 to 90% in the CA MRSA[102,103,104,110].

Binh An Diep et al 2004 described that pvl gene along with the type IV SCC mec element contributes to the spread of MRSA in the community, particularly in the skin and soft tissue infections.

22% of MRSA from Inpatients also were positive for pvl gene which was similar to the study by Binh An Diep et al 2004 which stated that the emergence of pvl type IV SCCmec MRSA strains in the community increases the concern that these strains would migrate into the hospital setting , and CA-MRSA strains which were susceptible to antibiotics other than the beta-lactams, would emerge as a multi drug resistant strains.[104,100,32] But our findings were in contrast to the studies by D'Souza N et al 2010 and other studies which showed 100% positivity of pvl gene in CA-MRSA and none of the HA-MRSA in these studies showed pvl gene[107,108,109,].

Susceptibility to Vancomycin was done by Macrobroth dilution method for all the MRSA isolates (TABLE22).All the isolates were found to be sensitive which was similar to the study by INSAR group[63],which states that MRSA isolates were more resistant as compared with the MSSA isolates and glycopeptides continue to remain the mainstay for treatment for MRSA infections. Hence glycopeptides should be preserved and encouraged to be used only in MRSA cases .

SUMMARY

- A total of 200 patients with Secondary bacterial infection of dermatological lesions were analysed during the one year study period.
- Out of 200 samples 63(31%) , 62(31%),52(26%) and 23(12%) samples were taken respectively from Atopic Dermatitis/Eczema, Pemphigus, Psoriasis and Leprosy with infected ulcer cases.
- Out of 200 samples 122 samples were taken from male patients and 78 from female patients.
- 114 (57%) cases were from In patients and 86(43%) cases were from out patients.
- Atopic dermatitis/Eczema was more common in younger age groups, Psoriasis and pemphigus were more common in the old and middle age groups respectively.
- 88% samples were culture positive and 12 % were culture negative.
- Among the various skin lesions the highest culture positivity rate was found in leprosy with infected ulcer(100%) followed by

Atopic Dermatitis/Eczema(93.7%) , pemphigus (93.5%) and psoriasis(69.2%) the least.

- Aerobic Gram positive organisms accounts for 59.9% followed by aerobic gram negative 37.26% and anaerobic organisms 2.83%.

- *Staphylococcus aureus* was the most commonly isolated organism in this study (50.47%), followed by enteric gram negative bacilli (23.59%), *Pseudomonas aeruginosa* (13.67%) and anaerobic organisms (2.83%).

- *Staphylococcus aureus* was the most commonly isolated organisms in both IP and OP cases. Enteric gram negative bacilli were more in IP cases than in OP cases.

- *Escherichia coli* was more commonly isolated from lower limbs. Anaerobic *Peptostreptococci* were isolated from head and neck and *Bacteroides* from lower limbs.

- Mixed infections were found highest in Pemphigus(25.80%) followed by, Leprosy with infected ulcer(17.39%), Psoriasis(15.38%),Atopic Dermatitis/Eczema(12.69%)

- *Staphylococcus aureus*(48.64%) was the most common organism in pemphigus followed by *Pseudomonas aeroginosa*

,Enterobacteriaceae , *Streptococcus pyogenes* and Anaerobic organisms.

- In Atopic Dermatitis/Eczema 61.19% of isolates were *Staphylococcus aureus* followed by *Streptococcus pyogenes*,Enterobacteriacea and anaerobic organisms.

- Most common isolate in Psoriasis was *Staphylococcus aureus* (63.63%) followed by Enteric gram negative bacilli and *Staphylococcus epidermidis*.

- *Pseudomonas aeruginosa* (48.1%) was the most common organism in leprosy with infected ulcer followed by Proteus vulgaris (22.2%).

- Blood culture from17 In patients, [11 from Pemphigus (1.77%) and 6 from Psoriasis (1.15%)] resulted in MRSA isolation from two cases of Pemphigus.

- All gram positive organisms were highly sensitive to amikacin and least sensitive to penicillin and cotrimoxazole.

- All gram negative bacilli were 100% sensitive to imipenem and piperacillin-tazobactum and showed higher rate of sensitivity to amikacin and least to ciprofloxacin.

- ESBL producers were 62.5%. All the ESBL producers were sensitive to Imipenem.
- Out of 107 *Staphylococcus aureus* 69(64.48%) were MSSA and 38(35.52%) were MRSA.
- MRSA strains were resistant to most of the routinely used antimicrobial agents than MSSA.
- All MRSA were positive for mecA gene.MRSA from OP patients(CA MRSA) showed higher positivity for Pvl gene 90% and 22% of MRSA from IP patients(HA MRSA) were positive for Pvl gene
- All the Methicillin resistant *Staphylococcus aureus* were sensitive to vancomycin.

CONCLUSION

In this cross sectional study conducted at the Institute of Microbiology, Rajiv Gandhi Government General Hospital aimed at isolating the bacteria associated with dermatological lesions had revealed that *Staphylococcus aureus* was the common pathogen in Atopic dermatitis/Eczema, Psoriasis, Pemphigus lesions, from all body sites with nearly equal prevalence in inpatients and outpatients and Pseudomonas aeruginosa in chronic ulcers of leprosy patients. The incidence of Enterobacteriaceae infection was more in inpatients with higher levels of ESBL production.CA MRSA and HA MRSA with higher rate of resistance to many routinely used antibiotics were isolated.

Hence bacterial culture and sensitivity of specimens from the secondarily infected skin lesions should be performed to confirm the bacterial etiology and to initiate effective antibiotic treatment so as to decrease the morbidity and mortality of these patients, that also limits the misuse of antimicrobials which would prevent the emergence of resistant bacterial strains in the hospital and the community.

FIGURE 1: INFECTED ULCER OF LEPROSY

FIGURE2:INFECTED PEMPHIGUS

LESION

FIGURE 3 : INFECTED ATOPIC DERMATITIS

FIGURE 4 : INFECTED PSORIASIS LESION

FIGURE5: DIRECT GRAM STAIN SHOWING PUS CELLS AND GRAM POSITIVE COCCI IN CLUSTERS

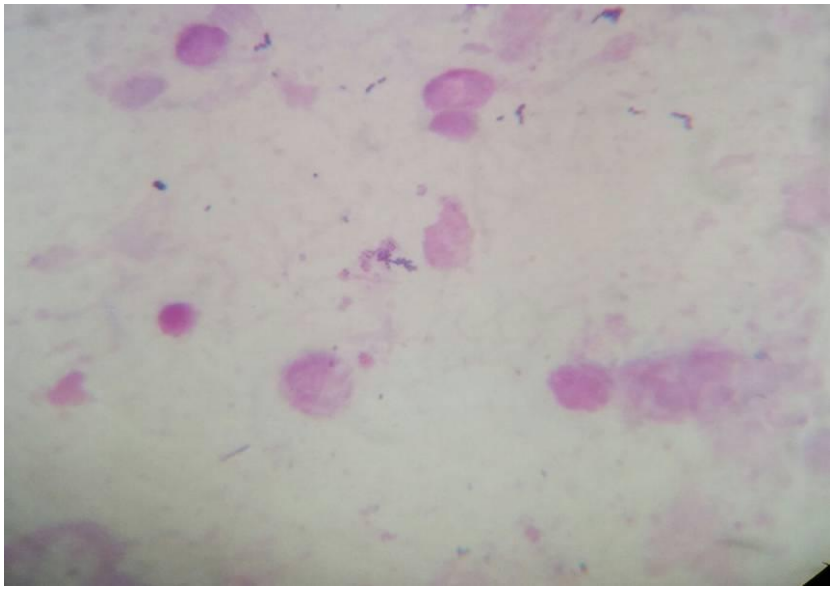


FIGURE 6: COLONIES OF *STREPTOCOCCUS PYOGENES* SHOWING BACITRACIN SENSITIVITY AND COTRIMOXAZOLE RESISTANCE

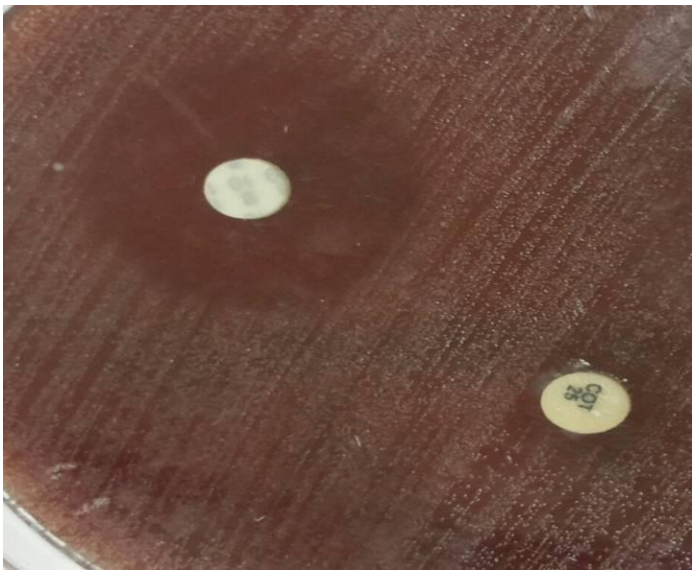


FIGURE 7: COLONIES OF *STAPHYLOCOCCUS AUREUS*

ON NUTRIENT AGAR PLATE

FIGURE 8:GRAM STAIN SHOWING GRAM POSITIVE COCCI

IN CLUSTERS

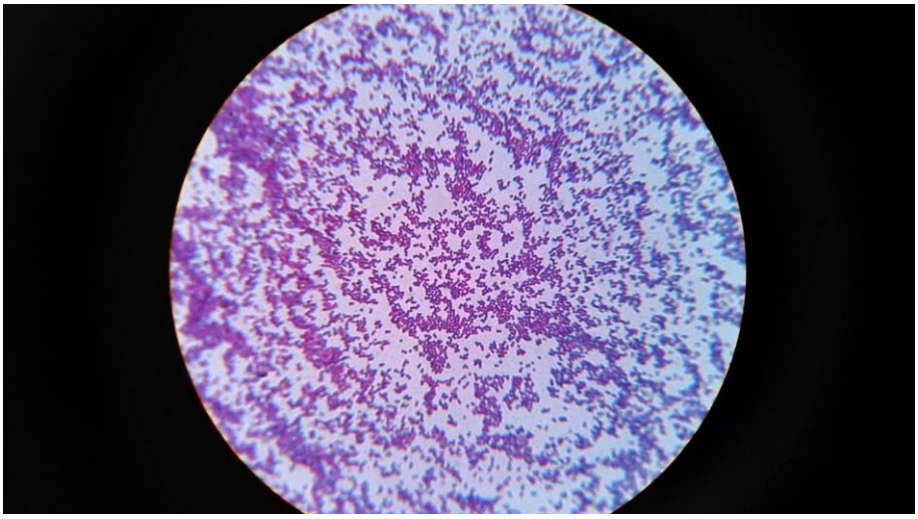


FIGURE9: TUBE COAGULASE TEST

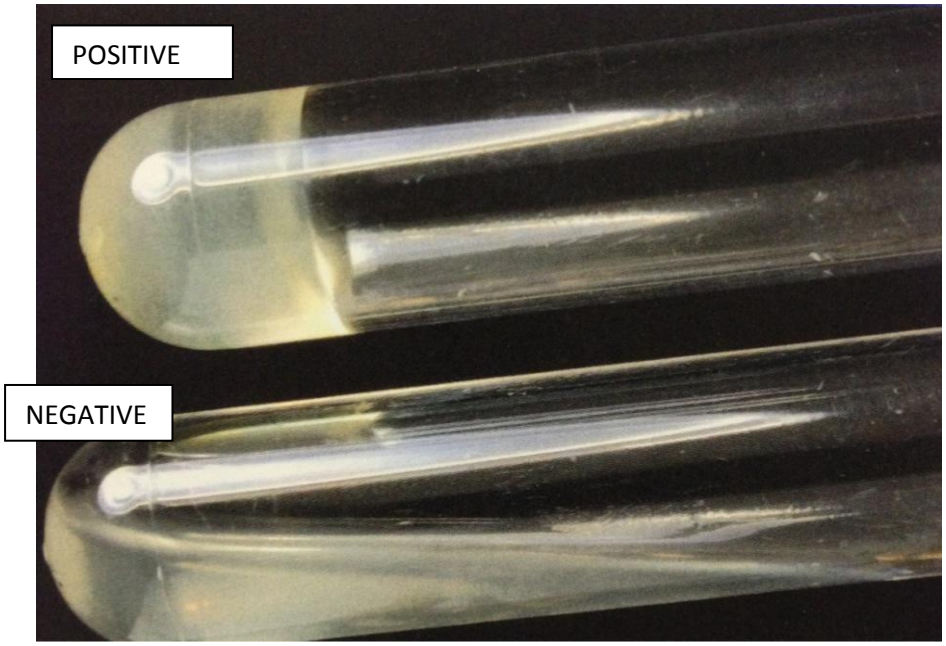


FIGURE 10: OXACILLIN MIC BY E-TEST FOR MRSA

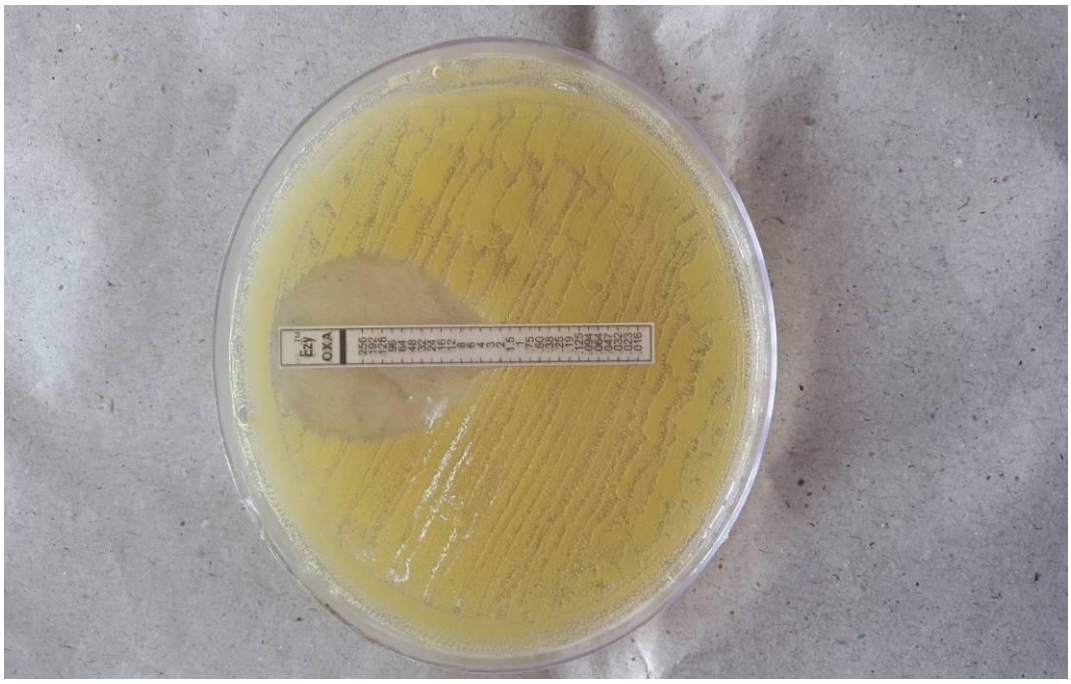


FIGURE 11:ANTIBIOGRAM OF MRSA SHOWING CEFOXITIN RESISTANCE

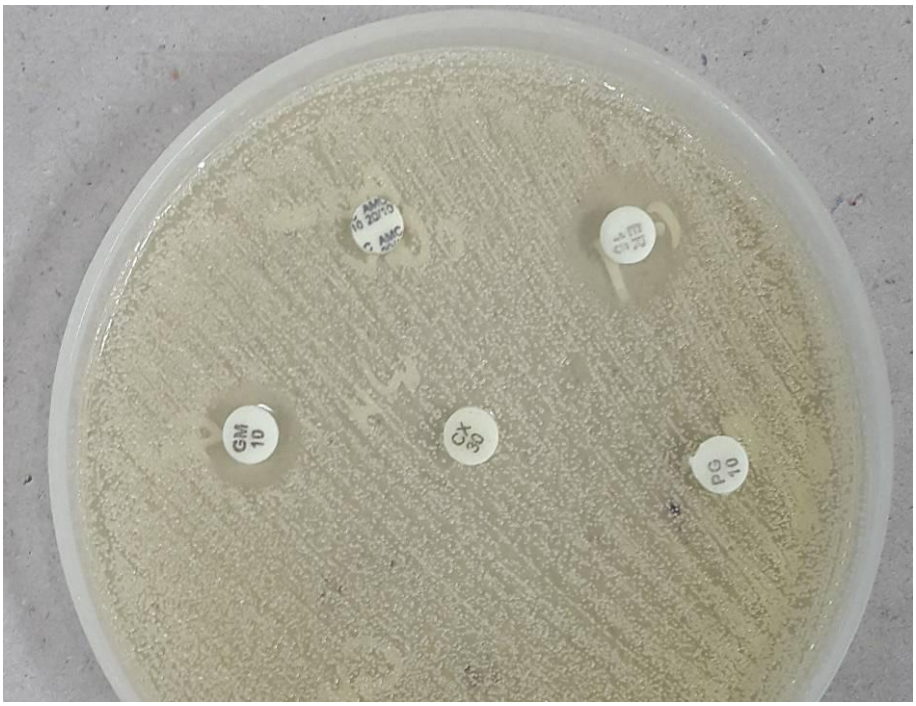


FIGURE 12: DETERMINATION OF VANCOMYCIN MIC FOR MRSA ISOLATES BY MACROBROTH DILUTION METHOD

FIGURE 13:DOUBLE DISC SYNERGY TEST (DDST)FOR ESBL PRODUCTION

FIGURE14:PHENOTYPIC CONFIRMATION DISC DIFFUSION TEST (PCDDT) FOR ESBL PRODUCTION

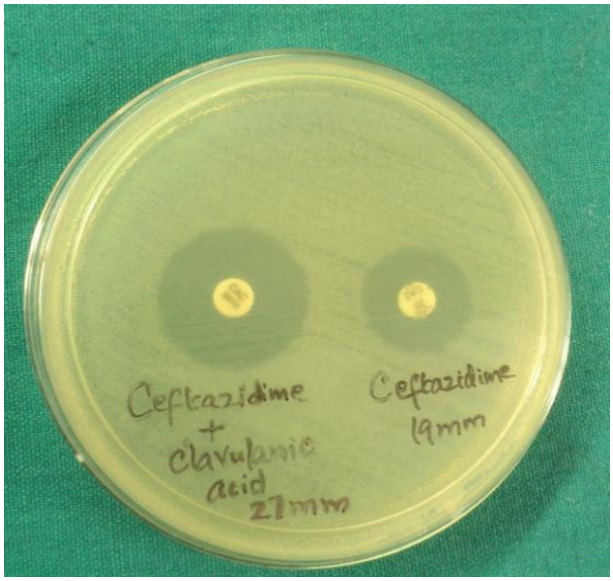


FIGURE15:MCINTOSH FILDES ANAEROBIC JAR AND GAS PAK

FIGURE 16: DNA EXTRACTION KIT

FIGURE 17: *Pvl* GENE DETECTION BY PCR

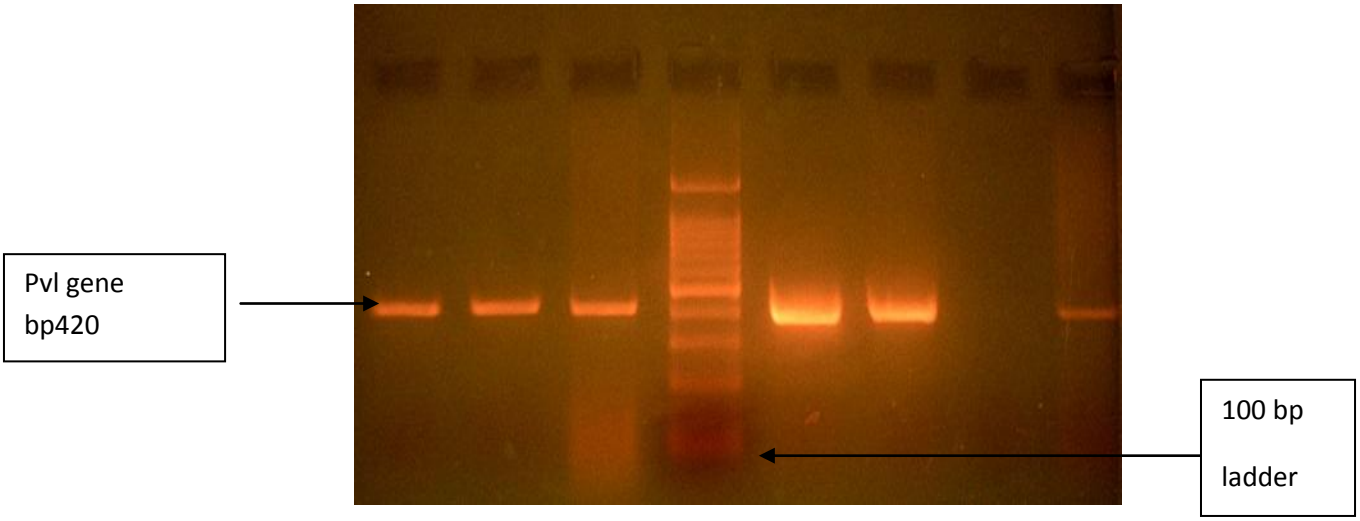
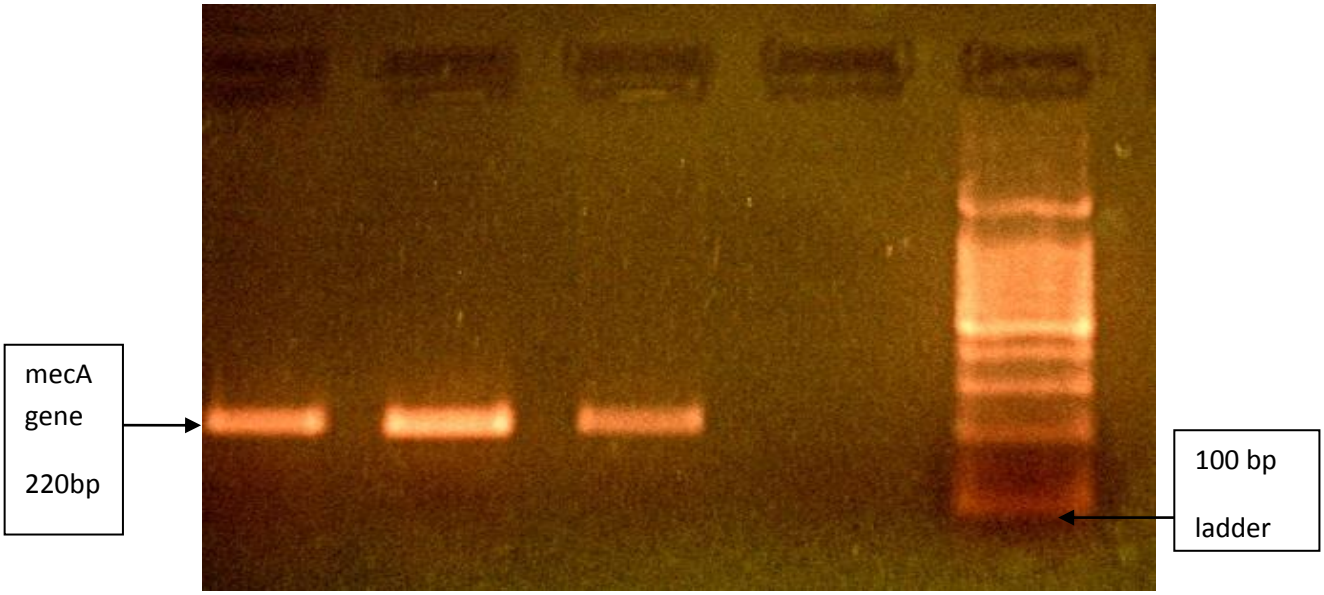


FIGURE 18:*mecA* GENE DETECTION BY PCR



APPENDIX – I

ABBREVIATIONS

SSTI	-	Skin and soft-tissue infection
TLR	-	Toll-like receptor
CMI	-	Cell-mediated immunity
PV	-	Pemphigus vulgaris
PF	-	Pemphigus foliaceus
ET	-	Exfoliative toxin
TSS	-	Toxic shock syndrome
CLSI	-	Clinical & Laboratory Standards Institute
ATCC	-	American Type Culture Collections
MIC	-	Minimum Inhibitory Concentration
MRSA	-	Methicillin Resistant Staphylococcus aureus
MSSA	-	Methicillin Sensitive staphylococcus aureus
CA-MRSA	-	Community acquired Methicillin Resistant Staphylococcus aureus
HA-MRSA	-	Hospital acquired Methicillin Resistant Staphylococcus aureus
SCC	-	Staphylococcal cassette chromosome
PVL	-	Panton Valentine leukocidin
PBP2a	-	Penicillin binding protein2a
RAPD	-	Random Amplified Polymorphic DNA
PCR	-	Polymerase chain reaction
RFLP	-	Restriction fragment length polymorphism
SSCP	-	Single strand conformation polymorphism
PCDDT	-	Phenotypic Confirmatory Disk Diffusion Test
DDST	-	Double Disc Synergy Test
ESBL	-	Extended Spectrum Beta Lactamase
MBL	-	Metallo BetaLactamase
IP	-	Inpatient
OP	-	Out patient

APPENDIX II

A. STAINS AND REAGENTS

1. Gram staining

Methyl violet (2%)	l0g Methyl violet in 100ml absolute alcohol in 1 litre of distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI (fixative)
Acetone	Decolourising agent
Carbol fuchsin 1%	Secondary stain.

B. MEDIA USED

1. Mac Conkey agar

Peptone	20g 5 g
Sodium taurocholate	1 ltr 20 g
Distilled Water	3.5ml
Agar	l00ml
2% neutral red in 50% ethanol	
10% lactose solution	

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix.Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

2. Nutrient agar

Peptic digest of animal tissue	5g
Sodium chloride	5g
Beef extract	1.5g
Yeast extract	1.5g

Agar	15gm

Final pH 7.4±0.2

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (120°C) for 15 minutes.

3. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	l0g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust pH to 7.4.

4. Chocolate agar

Sterile defibrinated blood	10 ml
Nutrient Agar (melted)	100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution.

5. **Cation adjusted Mueller- Hinton Agar**

Beef infusion	300ml
Caesein hydrolysate	17.5g
Starch	1.5g

Agar	l0g
Distilled water	lltr

pH = 7.4

Sterilise by autoclaving at 121°C for 20 mins

6. Robertson's Cooked Meat Broth

Fresh bullock heart	5 00g
Water	500ml
Sodium hydroxide, lmol/1	1.5ml
Liquid filtered from cooked meat	500ml
Peptone	2.5g
NaCl	1.25g

7. Selective Anaerobic Blood Agar:

1 µg/ml menadione and 20 µg/ml gentamicin added to the blood agar.

8. Thioglycollate broth

Pancreatic digest of casein	15gms
Yeast extract	5gms
Dextrose (Glucose)	5.5gms
Sodium chloride	2.5gms
L-Cystine	0.5gms

Autoclaved at 15 lbs pressure (121°C) for 20 minutes.

Note: If more than the upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating in a water bath or until the pink colour disappears.

C. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION

1. Oxidase Reagent

Tetra methyl p-phenylene diamine dihyrochloride- 1% aqueous solution.

2. Catalase

3% hydrogen peroxide

3. Indole test

Kovac's reagent

Amyl or isoamyl alcohol 150ml Para dimethyl amino benzaldehyde lOg Concentrated hydrochloric acid 50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4. Christensen's Urease test medium

Peptone	lg
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1 ltr

10% sterile solution of glucose	10ml
Sterile 20% urea solution	100ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

5. Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20 g
Bromothymol blue 0.2%	40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

6. Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	lg
Lactose	10 g
Sucrose	l0g
Ferric citrate	0.3g
Sodium chloride	5g
S odum thiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	12ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

7. Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr
Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter dispense in 5ml amounts and sterilize at 121°C for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube.

Methyl Red Reagent

Methyl Red	l0mg
Ethyl alcohol	30ml
Distilled water	20ml

Voges Proskauer Reagent

Reagent A: Alpha naphthol	5g
Ethyl alcohol	100ml
Reagent B: Potassium hydroxide	40g
Distilled water	100ml

8. Peptone water fermentation test medium

To the basal medium of peptone water, add sterilised sugars of 1% indicator bromothymol blue with Durham's tube. Basal medium peptone water Sugar solutions:

Sugar	1ml
Dislilled water	100ml

pH = 7.6.

9. Mannitol motility medium

Agar	5g
Peptone	lg
Potassium nitrate	1g
Mannitol	2g
Phenol red indicator	
Distilled water	1000ml
pH	7.2

10. Phenolphthalein diphosphate agar

- Sterilize a 1% aqueous solution of sodium phenolphthalein diphosphate by filtration and store at 4°C
- Add 10ml of this solution to 1000ml melted nutrient agar cooled to 50°C and pour plates
- Grow the staphylococcus overnight at 37°C on the medium
- Invert the plate and pour a few drops of ammonia solution SG 0.88 into the lid
- Read as positive a culture whose colonies turn bright pink within a few minutes. The colour soon fades.

11. Potassium nitrate broth

Potassium nitrate (KNO_3)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclaved.

12. Phenyl alanine deaminase test

Yeast Extract	3g
Dl-Phenylalamine	2 g
Disodium hydrogen phosphate	l g

Sodium Chloride	5 g
Agar	12g
Distilled water	1 lr
PH	7.4

Distributed in tubes and sterilized by autoclaving at 121° C for 1 5 minutes, allowed to solidify as long slopes.

13. Sugar fermentation medium

Peptone	15g
Andrade's indicator	10 ml
Sugar to be tested	20g
Water	1 litre

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1M sodium hydroxide has been added to turn the colour of the solution yellow.

Dissolve the peptone and Andrade's indicator in 1 litre of water and add 20g of the sugar; sugars to be tested generally include glucose, sucrose, lactose and maltose. Distribute 3ml amounts in standard test tubes containing an inverted Durham tube. Sterilize by steaming at 100 degree C for 30 min on 3 consecutive days.

ANNEXURE-I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr.S.Vinotha,
Post Graduate in MD Microbiology,
Institute of Microbiology,
Madras Medical College, Chennai-3.

Dear **Dr. S.Vinotha,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"A Study on Secondary Bacterial Infections in Dermatological Lesions and their Anti Microbial Susceptibility Pattern in a Tertiary care Hospital"** No.15122013

The following members of Ethics Committee were present in the meeting held on 11.12.2013 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS	-- Chairperson
2. Prof. B. Kalaiselvi, MD Vice Principal, MMC, Ch-3	-- Member Secretary
3. Prof. Ramadevi, Director i/c, Instt. of Biochemistry, Chennai.	-- Member
4. Prof. P. Karkuzhali, MD for Dr. V. Ramamoorthy Prof. Instt. of Pathology, MMC, Ch-3	-- Member
5. Thiru. S. Govindasamy, BABL	-- Lawyer
6. Tmt. Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

15/12/13

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE-II

PROFORMA

Name : IP/OP No:_____

Age : Ward:_____

Sex : **M / F**

Occupation:

Address:_____

_____

Presenting complaints:

- ✓ Skin lesions associated with pain and discharge

Local examination:

- ✓ Skin lesions with redness, swelling
- ✓ Nature of discharge-purulent/watery

Provisional diagnosis:

<u>Laboratory evaluation</u>

Microbiological investigation:

Sample collected:

- Swab from exudates of the lesion

Direct examination

Gram's Stain:

Bacterial Culture: MAC

BAP

CAP

Isolate identified in sample:

Antibacterial susceptibility pattern:

ANNEXURE-III

CONSENT FORM

STUDY TITLE: A study on secondary bacterial infections associated with dermatological lesions and their antimicrobial susceptibility pattern in a tertiary care hospital."

I……, hereby give consent to participate in the study conducted by Dr.S.Vinotha, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my sample for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression of the patient/ relative Place:

Patient Name & Address: Date:

Signature of the investigator **Signature of the guide:**

S.No	Age	Sex	IP/OP No	Wd	Skin Lesion	Site	fever	Immuno suppressants/ steroids	organism 1	organism 2	blood isolate	Amikacin	Cipro	cotri	chloramphenicol	Penicillin	erythro	Cefoxitin	Vancomycin	Amikacin	Cefotaxime	Ceftazidime	cotri	Cipro	Genta	imipenem	PT
1	72	M	IP112824	44	Psoriasis	Arm		Y	Staphylococcus aureus [MRSA]	N	N	S	R	R	R	R	R	R	S	N	N	N	N	N	N	N	N
2	72	F	IP117921	45	Pemphigus	Forearm	Y	Y	Staphylococcus aureus	N	NG	S	S	R	R	R	R	S	N	N	N	N	N	N	N	N	N
3	74	M	IP 118706	44	Atopic Dermatitis/Eczema	Left Leg		Y	Escheria coli	N	N	N	N	N	N	N	N	N	N	S	S	S	R	R	S	S	S
4	22	M	IP 114476	45	Atopic Dermatitis/Eczema	Right Leg		Y	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
5	57	M	IP 112126	44	Psoriasis	Right Hand		Y	Staphylococcus aureus [MRSA]	Escheria coli	N	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S
6	60	M	IP 120774	44	Atopic Dermatitis/Eczema	Right Foot		Y	Staphylococcus aureus	N	N	S	R	R	S	S	S	S	N	N	N	N	N	N	N	N	N
7	56	M	IP 120774	44	Psoriasis	Left Hand	Y	Y	Staphylococcus aureus	proteus vulgaris	NG	S	R	S	S	R	R	S	N	S	S	S	R	R	R	S	S
8	35	F	IP121843	45	Pemphigus	Forehead Scalp	Y	Y	Staphylococcus aureus [MRSA]		NG	R	R	R	R	R	R	R	S	N	N	N	N	N	N	N	N
9	55	M	OP 777212	-	Atopic Dermatitis/Eczema	Right Arm		N	CONS[MS]	N	N	S	S	S	S	R	R	S	N	N	N	N	N	N	N	N	N
10	35	M	OP 21382	-	Atopic Dermatitis/Eczema	Left Foot		N	Streptococcus pyogenes	N	N	S	R	R	S	S	S	N	N	N	N	N	N	N	N	N	N
11	53	F	OP26864	-	Atopic Dermatitis/Eczema	Left Foot		Y	Staphylococcus aureus	N	N	S	R	R	S	R	R	S	N	N	N	N	N	N	N	N	N
12	35	M	IP 110862	13	Leprosy Right Arm	Left Leg		N	Pseudomonas aeroginosa	N	N	N	N	N	N	N	N	N	N	S	S	S	R	R	R	S	S
13	84	F	IP 6102	45	Pemphigus	Back	Y	Y	Staphylococcus aureus [MRSA]	N	Staphylococcus aureus[MRSA]	R	R	R	R	R	R	R	S	N	N	N	N	N	N	N	N
14	20	M	IP 130684	44	Pemphigus	Back		Y	Staphylococcus aureus	Klebsiella pneumoniae[ESBL]	N	S	R	S	S	S	S	S	N	S	R	R	R	R	S	S	S
15	66	M	IP27252	44	Pemphigus	Left Leg		Y	Pseudomonas species	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	S	S	S
16	30	M	IP26292	44	Psoriasis	Scalp		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
17	52	M	IP95234	44	Psoriasis	Back		Y	CONS[MS]	Proteus mirabilis [ESBL]	N	S	S	S	S	S	S	S	N	S	R	R	R	R	R	S	S
18	60	F	OP25670	-	Atopic Dermatitis/Eczema	Hand		Y	Streptococcus pyogenes	N	N	S	S	R	R	S	S	N	N	N	N	N	N	N	N	N	N
19	65	M	OP21137	-	Atopic Dermatitis/Eczema	Right hand		N	CONS[MS]	N	N	S	R	R	S	R	S	S	N	N	N	N	N	N	N	N	N
20	62	M	OP24848	-	Atopic Dermatitis/Eczema	Elbow back		N	Staphylococcus aureus	Peptostreptococci	N	R	S	R	S	R	R	S	N	N	N	N	N	N	N	N	N
21	48	M	IP37962	44	Pemphigus	Back		Y	Escheria coli[ESBL]	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	S	S	S
22	50	F	IP37377	45	Pemphigus	Scalp		Y	Pseudomonas species	Peptostreptococci	N	N	N	N	N	N	N	N	N	S	R	R	R	R	R	S	S
23	40	F	IP38973	45	Pemphigus	UL + Back		Y	Staphylococcus aureus	Escheria coli[ESBL]	N	S	S	S	S	S	S	S	N	S	R	R	R	R	S	S	S
24	65	F	IP 33061	45	Pemphigus	Left Arm		Y	Staphylococcus aureu	N	N	S	R	R	R	R	R	S	N	N	N	N	N	N	N	N	N
25	27	F	IP40719	45	Pemphigus	Left Leg		Y	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
26	38	F	IP33000	45	Psoriasis	Back		Y	Pseudomonas aeroginosa	N	N	N	N	N	N	N	N	N	N	S	S	S	R	S	S	S	S
27	18	M	IP41482	44	Atopic Dermatitis/Eczema	Right Leg		Y	Staphylococcus aureus	Bacteroids	N	S	R	R	S	R	R	S	N	N	N	N	N	N	N	N	N
28	42	F	IP36221	45	Psoriasis	Left Leg	Y	Y	Pseudomonas species	N	NG	N	N	N	N	N	N	N	N	S	S	S	R	R	R	S	S
29	42	F	IP35670	45	Psoriasis	Leg		Y	Escheria coli[ESBL]	N	N	N	N	N	N	N	N	N	N	S	R	R	S	S	S	S	S
30	38	F	OP12186		Psoriasis	Back		Y	Staphylococcus aureus	N	N	S	S	S	S	R	S	S	N	N	N	N	N	N	N	N	N
31	42	M	OP21864		Atopic Dermatitis/Eczema	Left Leg		N	Staphylococcus aureus	Klebsiella oxytoca	N	S	S	S	S	S	S	S	N	S	S	S	R	R	R	S	S
32	63	M	OP31832		Pemphigus	Back		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
33	23	M	OP12186		Atopic Dermatitis/Eczema	Right Hand		N	Streptococcus pyogenes	N	N	S	R	R	S	S	S	N	N	N	N	N	N	N	N	N	N
34	55	M	IP110161		Psoriasis	Right hand		Y	Staphylococcus aureus	N	N	S	R	R	S	R	R	S	N	N	N	N	N	N	N	N	N
35	68	M	IP120614	44	Pemphigus	Right Arm back		Y	Staphylococcus aureus	Pseudomonas	N	S	S	S	S	S	S	S	N	R	S	S	R	S	R	S	S
36	59	F	IP110618	45	Psoriasis	Scalp		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
37	42	F	OP161721		Atopic Dermatitis/Eczema	Left Leg		N	Staphylococcus aureus	Escheria coli[ESBL]	N	S	R	R	S	R	R	S	N	S	R	R	S	S	S	S	S
38	28	M	IP11681		Atopic Dermatitis/Eczema	Right foot		N	Staphylococcus aureus	N	N	R	R	S	S	R	R	S	N	N	N	N	N	N	N	N	N
39	59	M	IP11863		Pemphigus	Back Forehead		Y	Klebsiella oxytoca [ESBL]	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	S	S	S
40	28	F	IP12823		Atopic Dermatitis/Eczema	Right hand		Y	Staphylococcus aureus [MRSA]	N	N	R	R	R	R	R	R	R	S	N	N	N	N	N	N	N	N
41	42	F	IP11682		Pemphigus	Back Left Leg	Y	Y	Escheria coli	N	NG	N	N	N	N	N	N	N	N	S	S	S	R	R	S	S	S
42	58	M	IP110714		Pemphigus	Right Leg Forehead	Y	Y	Staphylococcus aureus[MRSA]	N	NG	S	R	R	S	R	R	R	S	N	N	N	N	N	N	N	N
43	63	F	IP11191		Psoriasis	Chest	Y	Y	Staphylococcus aureus	N	NG	R	S	R	S	S	S	S	N	N	N	N	N	N	N	N	N
44	39	M	OP31432		Psoriasis	Left Hand Back		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
45	28	M	OP31131		Leprosy Right Arm	Right Foot		N	Proteus vulgaris	N	N	N	N	N	N	N	N	N	N	S	S	S	R	R	R	S	S
46	34	M	HNO 98/14 OP 32141		Leprosy Right Arm	Left Hand		N	Pseudomanas species	N	N	N	N	N	N	N	N	N	N	R	S	S	R	S	S	S	S
47	60	F	OP41361		Psoriasis	Buttock		Y	No Growth	N	N	N	N	N	N	N	N	N	N								
48	41	M	IP12361		Leprosy Right Arm	Right Arm		N	Klebsiella oxytoca [ESBL]	N	N	N	N	N	N	N	N	N	N	R	R	R	R	R	R	S	S
49	29	M	IP120161		Atopic Dermatitis/Eczema	Right hand		Y	Staphylococcus aureus	N	N	S	S	R	R	R	R	S	N	N	N	N	N	N	N	N	N
50	23	F	Lep. No.112/14 O.P.30132		Leprosy with cellulitis Lt.leg	Left Foot		N	Proteus vulgaris	N	N	N	N	N	N	N	N	N	N	S	S	S	R	R	R	S	S
51	32	M	H.No.56/14 OP 31511		Leprosy bb	Right Leg		N	Staphylococcus aureus	Pseudomanas species	N	S	R	R	R	S	S	S	N	S	R	R	R	R	R	S	S
52	50	M	IP 130121		Pemphigus	Right Leg		Y	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
53	57	M	IP64542		Psoriasis	left leg		Y	Staphylococcus aureus	Proteus vulgaris	N	R	S	R	S	R	R	S	N	R	S	S	R	R	S	S	S
54	45	M	OP52045		Atopic Dermatitis/Eczema	Right foot		N	Staphylococcus aureus	N	N	S	S	R	S	S	R	S	N	N	N	N	N	N	N	N	N
55	60	F	65692		Psoriasis	Left hand		Y	Staphylococcus aureus	N	N	S	R	R	R	R	R	S	N	N	N	N	N	N	N	N	N
56	65	F	IP67600		Pemphigus	Back		Y	CONS[MS]	N	N	S	R	R	S	S	S	S	N	N	N	N	N	N	N	N	N
57	47	F	OP H.No.325/14		Leprosy Right Arm	Left leg		N	Pseudomanas species	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	R	S	S
58	50	M	IP62146		Pemphigus	Left leg		Y	Staphylococcus aureus	Pseudomanas species	N	S	R	S	S	R	S	S	N	S	S	S	R	S	S	S	S
59	13	M	O.P 34088		Atopic Dermatitis/Eczema	Right Arm		N	Staphylococcus aureus	N	N	R	R	S	S	S	S	S	N	N	N	N	N	N	N	N	N
60	53	M	IP 68411		Pemphigus	Back		Y	Staphylococcus aureus	Streptococcus pyogenes	N	S	S	R	S	S	S	S	N	N	N	N	N	N	N	N	N
61	25	M	OP40812		Atopic Dermatitis/Eczema	Right Hand		N	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
62	45	M	OP31087		Atopic Dermatitis/Eczema	Left leg		N	Klebsiella oxytoca[ESBL]	N	N	N	N	N	N	N	N	N	N	S	R	R	S	R	R	S	S
63	28	F	OP41812		Atopic Dermatitis/Eczema	Right arm		N	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
64	65	F	IP63545		Psoriasis	Back		Y	Staphylococcus aureus	N	N	S	S	S	S	R	R	S	N	N	N	N	N	N	N	N	N
65	70	F	IP 51236		Pemphigus	Left leg	Y	Y	Escheria coli [ESBL]	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	R	S	S
66	50	M	OP41713		Pemphigus	Left leg		Y	Klebsiella pneumoniae	N	N	N	N	N	N	N	N	N	N	R	S	S	R	R	R	S	S
67	60	M	OP31267		Psoriasis	right forearm		Y	Staphylococcus aureus	N	N	R	R	R	S	S	S	S	N	N	N	N	N	N	N	N	N
68	48	M	H.No.126/14		Leprosy Right Arm	Right leg		N	Proteus vulgaris	N	N	N	N	N	N	N	N	N	N	S	S	S	R	R	R	S	S
69	51	M	H.No.113/13		Leprosy Right Arm	Left Foot		N	Pseudomonas	N	N	N	N	N	N	N	N	N	N	S	S	S	R	S	S	S	S
70	25	M	OP51013		Atopic Dermatitis/Eczema	Buttock		N	Escheria coli	N	N	N	N	N	N	N	N	N	N	S	S	S	S	S	S	S	S
71	38	M	OP41715		Atopic Dermatitis/Eczema	Righthand		N	Staphylococcus aureus[MRSA]	N	N	S	R	R	S	R	R	R	S	N	N	N	N	N	N	N	N

72	39	F	IP43416		Pemphigus	Righthand		Y	Staphylococcus aureus[MRSA]	N	N	S	R	R	R	R	R	R	S	N	N	N	N	N	N	N	N
73	40	F	51216		Psoriasis	Right arm		Y	Staphylococcus aureus	N	N	S	R	R	S	S	S	S	N	N	N	N	N	N	N	N	N
74	29	F	51315		Atopic Dermatitis/Eczema	Left arm		N	Staphylococcus aureus	N	N	S	S	S	S	R	R	S	N	N	N	N	N	N	N	N	N
75	65	F	IP61234		Psoriasis	Fore head		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
76	70	M	IP51651		Pemphigus	Left Leg		Y	Staphylococcus aureus	Pseudomonas	N	S	S	R	S	R	R	S	N	N	N	N	N	N	N	N	N
77	40	M	IP112621	45	Pemphigus	Back		Y	Staphylococcus aureus[MRSA]	N	N	R	R	R	R	R	R	R	S	N	N	N	N	N	N	N	N
78	38	M	OP31267		Atopic Dermatitis/Eczema	Right Hand		N	Staphylococcus aureus[MRSA]	N	N	S	R	R	R	R	R	R	S	N	N	N	N	N	N	N	N
79	43	M	OP57313		Atopic Dermatitis/Eczema	Left Arm		N	Staphylococcus aureus	N	N	R	S	S	S	R	S	S	N	N	N	N	N	N	N	N	N
80	65	M	IP113217		Psoriasis	Right Hand		Y	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
81	35	F	OP31337		Atopic Dermatitis/Eczema	Right leg		Y	Staphylococcus aureus [MRSA]	N	N	S	S	R	S	S	R	R	S	N	N	N	N	N	N	N	N
82	68	F	IP116532		Pemphigus	Back	Y	Y	Klebsiella pneumoniae [ESBL]	N	NG	N	N	N	N	N	N	N	N	S	R	R	R	R	S	S	S
83	56	F	IP51321		Pemphigus	Right Hand		Y	Staphylococcus aureus [MRSA]	N	N	R	R	R	S	R	R	R	S	N	N	N	N	N	N	N	N
84	60	F	IP116113		Psoriasis	Left leg		Y	Staphylococcus aureus	N	N	R	S	S	S	R	R	S	N	N	N	N	N	N	N	N	N
85	39	M	OP32138		Atopic Dermatitis/Eczema	Right hand		N	Streptococcus pyogenes	N	N	S	S	R	S	S	S	N	N	N	N	N	N	N	N	N	N
86	32	M	OP41211		Atopic Dermatitis/Eczema	Right arm		N	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
87	68	M	IP112812		Pemphigus	Left leg		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
88	58	M	Op 51312		Psoriasis	Left hand		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
89	28	M	OP31618		Atopic Dermatitis/Eczema	Right leg		N	Staphylococcus aureus[MRSA]	N	N	S	R	R	S	R	R	R	S	N	N	N	N	N	N	N	N
90	40	F	OP51218		Atopic Dermatitis/Eczema	left thigh		N	Staphylococcus aureus	N	N	S	R	R	R	S	R	S	N	N	N	N	N	N	N	N	N
91	54	M	IP116181		Pemphigus	Back		Y	Staphylococcus aureus [MRSA]	N	N	S	S	R	S	R	R	R	S	N	N	N	N	N	N	N	N
92	48	M	OP31241		Psoriasis	Left leg		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
93	32	F	OP21318		Atopic Dermatitis/Eczema	Right leg		N	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
94	40	F	OP41216		Psoriasis	Left hand		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
95	50	M	Lep no221/13		Leprosy Right Arm	Right Hand		N	Klebsiella oxytoca[ESBL]	Escheria coli	N	N	N	N	N	N	N	N	N	R/S	R/S	R/S	R/S	S/S	S/S	S/S	S/S
96	80	F	IP63646		Pemphigus	Left leg		Y	Staphylococcus aureus	N	N	S	R	R	R	R	R	S	N	N	N	N	N	N	N	N	N
97	72	M	IP61411		Psoriasis	Right hand		Y	Staphylococcus aureus	N	N	S	R	S	S	S	R	S	N	N	N	N	N	N	N	N	N
98	62	F	Lep. No.106/13 O.P.31816		Leprosy Right Arm	Right foot		N	Pseudomonas aeroginosa	N	N	N	N	N	N	N	N	N	N	R	R	R	R	R	R	S	S
99	80	M	IP16122		Pemphigus	Fore head		Y	CONS[MS]	Pseudomonas	N	S	R	S	S	S	S	S	N	S	S	S	S	S	S	S	S
100	69	F	IP31244		Pemphigus	Right leg		Y	Klebsiella oxytoca [ESBL]	N	N	N	N	N	N	N	N	N	N	R	R	R	R	R	R	S	S
101	82	M	Lep. No.126/13 O.P.32114		Leprosy Left foot	Left Foot		N	Pseudomonas	N	N	N	N	N	N	N	N	N	N	S	S	S	R	S	S	S	S
102	46	M	Lep. No.116/13 O.P.12113		Leprosy Right Arm	Left Foot		N	Proteus vulgaris	N	N	N	N	N	N	N	N	N	N	S	S	S	S	R	R	S	S
103	61	F	Lep. No.131/13 O.P.41236		Leprosy Right Arm	Right Leg		N	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	S	R	R	R	R	R	S	S
104	46	F	IP31621		Pemphigus	Right Leg		Y	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
105	53	M	OP51617		Psoriasis	left leg	Y	Y	Staphylococcus aureus	Proteus vulgaris	NG	S	S	R	S	R	R	S	N	R	S	S	R	R	R	S	S
106	25	M	IP63984		Atopic Dermatitis/Eczema	Right foot		Y	Staphylococcus aureus	N	N	R	S	S	S	R	R	S	N	N	N	N	N	N	N	N	N
107	43	M	IP41651		Psoriasis	Left hand		Y	Staphylococcus aureus	N	N	S	R	R	R	R	R	S	N	N	N	N	N	N	N	N	N
108	69	F	IP122621		Pemphigus	Back		Y	CONS[MS]	N	N	S	S	R	R	R	S	S	N	N	N	N	N	N	N	N	N
109	49	M	OP21267		Leprosy Right Arm	Left leg		N	Pseudomanas species	N	N	N	N	N	N	N	N	N	N	S	R	S	S	S	R	S	S
110	53	M	OP56313		Pemphigus	Left leg		Y	Staphylococcus aureus	Pseudomanas species	N	S	R	R	R	S	R	S	N	S	S	S	R	S	S	S	S
111	43	M	IP112117		Atopic Dermatitis/Eczema	Right Arm		Y	Staphylococcus aureus	N	N	S	S	S	S	R	S	S	N	S	S	S	S	S	S	S	S
112	66	F	OP31427		Pemphigus	Back		N	Staphylococcus aureus	Streptococcus pyogenes		S/S	S/S	S/R	S/S	R/S	R/S	S/N	N/N	N	N	N	N	N	N	N	N
113	47	M	IP116521		Psoriasis	Right Hand		Y	Staphylococcus aureus [MRSA]	N	N	S	R	R	R	R	R	R	S	N	N	N	N	N	N	N	N
114	32	M	IP51091		Atopic Dermatitis/Eczema	Right leg		Y	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
115	78	M	IP112313		Pemphigus	Back		Y	Klebsiella pneumoniae	N	N	N	N	N	N	N	N	N	N	S	S	S	S	S	S	S	S
116	55	M	OP12138		Pemphigus	Right Hand		Y	Staphylococcus aureus	N	N	S	S	R	S	S	S	S	N	N	N	N	N	N	N	N	N
117	71	M	OP40611		Psoriasis	Left leg		Y	Staphylococcus aureus	N	N	S	S	R	R	R	S	S	N	N	N	N	N	N	N	N	N
118	47	F	IP101812		Atopic Dermatitis/Eczema	Right hand		Y	Streptococcus pyogenes	N	N	S	R	R	S	S	R	N	N	N	N	N	N	N	N	N	N
119	33	M	Op 31312		Atopic Dermatitis/Eczema	Right arm		N	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
120	42	M	IP21973		Pemphigus	Left leg		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
121	56	F	IP 31061		Psoriasis	Left hand		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
122	51	M	IP40419		Atopic Dermatitis/Eczema	Right leg		N	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
123	62	F	IP33042		Atopic Dermatitis/Eczema	left thigh		Y	Staphylococcus aureus	N	N	S	S	R	R	R	R	S	N	N	N	N	N	N	N	N	N
124	67	M	IP41422		Psoriasis	Arm		Y	Staphylococcus aureus [MRSA]	N	N	S	R	R	R	R	R	R	S	N	N	N	N	N	N	N	N
125	66	M	IP36311		Pemphigus	Forearm	Y	Y	Staphylococcus aureus	N	NG	R	R	R	R	R	R	R	S	N	N	N	N	N	N	N	N
126	32	M	IP35661		Atopic Dermatitis/Eczema	Left Leg		Y	Escheria coli	N	N	N	N	N	N	N	N	N	S	S	S	S	R	R	S	S	S
127	26	M	OP12176		Atopic Dermatitis/Eczema	Right Leg		N	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
128	50	F	OP21314		Psoriasis	Right Hand		Y	Staphylococcus aureus [MRSA]	Escheria coli	N	S	R	R	R	R	R	R	S	S	S	S	R	R	S	S	S
129	29	M	OP31830		Atopic Dermatitis/Eczema	Right Foot		N	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
130	47	M	OP12176		Psoriasis	Left Hand	Y	Y	Staphylococcus aureus	proteus vulgaris	NG	S	S	S	S	R	S	S	N	S	S	S	R	R	S	S	S
131	52	M	IP110123		Pemphigus	Forehead Scalp	Y	Y	Staphylococcus aureus [MRSA]	N	NG	S	S	S	S	R	R	R	S	N	N	N	N	N	N	N	N
132	42	F	IP110614		Atopic Dermatitis/Eczema	Right Arm		Y	CONS[MS]	N	N	S	R	R	S	S	S	N	N	N	N	N	N	N	N	N	N
133	34	F	IP110718		Atopic Dermatitis/Eczema	Left Foot		Y	Streptococcus pyogenes	N	N	S	S	R	S	S	S	N	N	N	N	N	N	N	N	N	N
134	65	F	OP151721		Psoriasis	Back		Y	Staphylococcus aureus [MRSA]	N	N	R	R	R	S	R	R	R	S	N	N	N	N	N	N	N	N
135	43	F	IP11641		Atopic Dermatitis/Eczema	Left Leg		Y	Staphylococcus aureus	Klebsiella oxytoca [ESBL]	N	S	S	S	S	R	S	S	N	S	R	R	R	R	R	S	S
136	49	F	IP12863		Pemphigus	Back		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
137	40	M	IP12893		Atopic Dermatitis/Eczema	Right Hand		Y	Streptococcus pyogenes	N	N	S	S	R	S	S	S	N	N	N	N	N	N	N	N	N	N
138	50	F	IP11582		Psoriasis	Right hand		Y	Staphylococcus aureus	N	N	S	R	S	S	R	R	S	N	N	N	N	N	N	N	N	N
139	56	F	IP109714		Pemphigus	Right Arm back		Y	Staphylococcus aureus [MRSA]	Pseudomonas	N	S	R	S	S	R	R	R	S	R	S	S	R	S	S	S	S
140	66	F	IP111919		Psoriasis	Scalp		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
141	33	M	OP31222		Atopic Dermatitis/Eczema	Left Leg		N	Staphylococcus aureus	Escheria coli	N	S	S	R	R	R	R	S	N	S	S	S	S	S	S	S	S
142	25	M	OP31061		Atopic Dermatitis/Eczema	Right foot		N	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N
143	47	M	IP123211		Pemphigus	Back Forehead		Y	Klebsiella oxytoca [ESBL]	N	N	N	N	N	N	N	N	N	N	R	R	R	R	R	R	S	S
144	34	M	OP41231		Atopic Dermatitis/Eczema	Right hand		N	Staphylococcus aureus [MRSA]	N	N	S	R	R	R	R	R	R	S	N	N	N	N	N	N	N	N
145	76	M	IP143001		Pemphigus	Back		Y	Staphylococcus aureus	N	N	S	S	R	S	S	R	S	N	N	N	N	N	N	N	N	N
146	56	F	OP 51321		Psoriasis	Left leg		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

147	23	F	OP 32021		Atopic Dermatitis/Eczema	Right leg		N	Staphylococcus aureus [MRSA]	N	N	S	S	S	S	S	S	R	S	N	N	N	N	N	N	N
148	45	M	OP61231		Psoriasis	Left hand		N	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
149	66	M	Lep. No.86/13 O.P.10013		Leprosy Right Arm	Right Hand		N	Klebsiella oxytoca[ESBL]	Escheria coli	N	N	N	N	N	N	N	N	S	R/S	R/S	R/S	S/S	S/S	S/S	S/S	S/S
150	69	F	OP 67123		Pemphigus	Left leg		Y	Staphylococcus aureus [MRSA]	N	N	S	R	R	S	R	R	R	S	N	N	N	N	N	N	N
151	60	F	OP56712		Psoriasis	Right hand		Y	Staphylococcus aureus [MRSA]	N	N	S	R	R	R	R	R	R	S	N	N	N	N	N	N	N
152	59	M	Lep. No.96/13 O.P.10213		Leprosy Right Arm	Right foot		N	Pseudomonas aeroginosa	N	N	N	N	N	N	N	N	N	N	R	R	R	R	R	R	S
153	71	F	IP110674		Pemphigus	Fore head		Y	CONS (MS)	Pseudomonas	N	S	S	R	R	R	S	S	S	S	R	R	R	R	R	S
154	64	M	IP210451		Pemphigus	Right leg		Y	Klebsiella oxytoca [ESBL]	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	S	S
155	43	M	OP31241		Atopic Dermatitis/Eczema	Left Foot		N	Staphylococcus aureus[MRSA]	N	N	S	S	S	S	R	R	R	S	N	N	N	N	N	N	N
156	69	M	Lep. No.102/13 O.P.10113		Leprosy Right Arm	Left Leg		N	Pseudomonas aeroginosa	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	R	S
157	58	F	IP 456123		Pemphigus	Back	Y	Y	Staphylococcus aureus [MRSA]	N	Staphylococcus aureus[MRSA]	S	R	R	R	R	R	R	S	N	N	N	N	N	N	N
158	56	M	IP116721		Pemphigous Vulgaris	Back		Y	Staphylococcus aureus [MRSA]	Klebsiella pneumoniae[ESBL]	N	S	S	R	S	R	R	R	S	S	R	R	R	R	R	S
159	36	M	IP 100237		Pemphigus	Left Leg		Y	Pseudomonas species	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	S	S
160	66	F	OP 32145		Psoriasis	Scalp		N	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
161	36	M	OP 42131		Psoriasis	Back		N	CONS[MS]	Proteus mirabilis [ESBL]	N	S	S	S	S	S	S	S	N	R	R	R	R	R	R	S
162	36	M	OP21321		Atopic Dermatitis/Eczema	Hand		N	Streptococcus pyogenes	N	N	S	S	R	S	S	S	N	N	N	N	N	N	N	N	N
163	29	M	OP28291		Atopic Dermatitis/Eczema	Right hand		N	CONS[MS]	N	N	R	S	R	S	S	S	S	N	N	N	N	N	N	N	N
164	34	M	IP121109		Atopic Dermatitis/Eczema	Elbow back		Y	Staphylococcus aureus [MRSA]	Peptostreptococci	N	R	S	R	S	R	R	R	S	N	N	N	N	N	N	N
165	46	F	IP291231		Atopic Dermatitis/Eczema	Buttock		Y	Escheria coli	N	N	S	S	R	S	S	R	R	S	N	N	N	N	N	N	N
166	44	F	OP 31243		Atopic Dermatitis/Eczema	Righthand		N	Staphylococcus aureus[MRSA]	N	N	S	R	R	R	R	R	R	S	N	N	N	N	N	N	N
167	48	F	IP238761		Pemphigus	Righthand		Y	Staphylococcus aureus[MRSA]	N	N	R	R	R	S	S	S	S	N	N	N	N	N	N	N	N
168	42	M	OP21323		Psoriasis	Right arm		Y	Staphylococcus aureus [MRSA]	N	N	R	R	R	R	R	R	R	S	N	N	N	N	N	N	N
169	25	M	OP15642		Atopic Dermatitis/Eczema	Left arm		N	Staphylococcus aureus[MRSA]	N	N	S	R	R	S	R	S	R	S	N	N	N	N	N	N	N
170	59	M	IP 231212		Psoriasis	Fore head		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
171	57	M	IP211345		Pemphigus	Left Leg		Y	Staphylococcus aureus	Pseudomonas	N	S	R	R	S	R	R	S	N	S	S	S	R	R	S	S
172	67	M	OP21312		Pemphigus	Back		Y	Staphylococcus aureus[MRSA]	N	N	R	R	R	R	R	R	R	S	N	N	N	N	N	N	N
173	37	F	OP37869		Atopic Dermatitis/Eczema	Right Hand		N	Staphylococcus aureus[MRSA]	N	N	S	S	R	R	R	R	R	S	N	N	N	N	N	N	N
174	23	F	OP54321		Atopic Dermatitis/Eczema	Left Arm		N	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N
175	66	F	IP110987		Pemphigus	Back		Y	Escheria coli	N	N	N	N	N	N	N	N	N	N	S	S	S	S	S	S	S
176	80	M	IP132411		Bullous Pemphigoid	Scalp		Y	Pseudomonas species	Peptostreptococci	N	N	N	N	N	N	N	N	N	S	S	S	R	R	R	S
177	79	M	IP 123754		Pemphigus	UL + Back		Y	Staphylococcus aureus	Escheria coli	N	S	S	R	R	R	R	R	S	N	S	S	S	R	S	S
178	58	M	OP21321		Pemphigus	Left Arm		Y	Staphylococcus aureus[MRSA]	N	N	R	R	R	R	R	R	R	S	N	N	N	N	N	N	N
179	61	M	IP26754		Pemphigus	Left Leg		Y	Staphylococcus aureus	N	N	S	S	R	R	S	S	S	N	N	N	N	N	N	N	N
180	59	M	IP217611		Psoriasis	Back		Y	Pseudomonas aeroginosa	N	N	N	N	N	N	N	N	N	N	S	S	S	R	S	S	S
181	45	M	IP 100312		Atopic Dermatitis/Eczema	Right Leg		N	Staphylococcus aureus	Bacteroids	N	S	S	S	S	R	R	S	N	N	N	N	N	N	N	N
182	58	F	IP129810		Psoriasis	Left Leg	Y	Y	Pseudomonas species	N	NG	N	N	N	N	N	N	N	N	R	S	S	R	R	R	S
183	71	F	OP 32785		Psoriasis	Leg		Y	Escheria coli	N	N	N	N	N	N	N	N	N	N	S	S	S	S	S	S	S
184	48	F	OP39870		Atopic Dermatitis/Eczema	Right Hand		N	Staphylococcus aureus [MRSA]	N	N	S	R	R	S	S	S	R	S	N	N	N	N	N	N	N
185	34	F	OP53421		Atopic Dermatitis/Eczema	Left leg		N	Klebsiella oxytoca	N	N	N	N	N	N	N	N	N	N	S	S	S	R	S	R	S
186	33	M	OP36712		Atopic Dermatitis/Eczema	Right arm		N	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
187	45	M	OP29871		Psoriasis	Back		Y	Staphylococcus aureus	N	N	S	R	R	S	S	S	S	N	N	N	N	N	N	N	N
188	69	M	IP110981		Pemphigus	Left leg		Y	Escheria coli[ESBL]	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	S	S
189	60	M	IP217631		Pemphigus	Left leg		Y	Klebsiella pneumoniae[ESBL]	N	N	S	S	S	S	S	S	S	N	R	S	S	R	S	R	S
190	58	M	OP21312		Psoriasis	right forearm		Y	Staphylococcus aureus	N	N	S	R	R	S	S	S	S	N	N	N	N	N	N	N	N
191	49	F	Lep. No.106/13 O.P.11323		Leprosy Right Arm	Right leg		N	Proteus vulgaris	N	N	N	N	N	N	N	N	N	N	R	R	R	R	R	R	S
192	69	F	Lep. No.76/13 O.P.10113		Leprosy Right Arm	Left Foot		N	Pseudomonas	N	N	N	N	N	N	N	N	N	N	S	S	S	R	R	R	S
193	60	F	IP100932		Pemphigus	Back Left Leg		Y	Escheria coli [ESBL]	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	S	S
194	59	F	IP231321		Pemphigus	Right Leg Forehead		Y	Staphylococcus aureus[MRSA]	N	N	S	R	R	R	R	R	R	S	N	N	N	N	N	N	N
195	66	M	OP13423		Psoriasis	Chest		N	Staphylococcus aureus	N	N	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N
196	59	M	OP34213		Psoriasis	Left Hand Back		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
197	56	M	Lep. No.116/13 O.P.12113	56/14 IP130981	Leprosy Right Arm	Right Foot		N	Proteus vulgaris	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	S	S
198	47	M	Lep. No.116/13 O.P.12113		Leprosy Right Arm	Left Hand		N	Pseudomanas species	N	N	N	N	N	N	N	N	N	N	S	S	S	S	S	S	S
199	39	F	OP321512		Psoriasis	Buttock		Y	No Growth	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
200	36	M	Lep. No.116/13 O.P.12113		Leprosy Right Arm	Right Arm		N	Klebsiella oxytoca[ESBL]	N	N	N	N	N	N	N	N	N	N	S	R	R	R	R	S	S

KEY TO MASTER CHART

M	Male
F	Female
Y	Yes
N	No
Cipro	Ciprofloxacin
Cotri	Cotrimoxazole
Erythro	Erythromycin
Genta	Gentamicin
PT	Piperacillin-tazobactum
R	Resistant
S	Sensitive
N	Not done

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